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ORGANIC CHEMICAL FATE PREDICTION IN ACTIVATED SLUDGE TREATMENT PROCESSES

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16. ABSTRACT

This report describes results from a broadly-based effort to determine the feasibility of predicting the fates of organic chemicals in diffused air, activated sludge wastewater treatment processes. The three conversion/removal mechanisms emphasized in this work were stripping, sorption on biomass, and bio-oxidation (biotransformation and mineralization). After an initial literature review and critique, separate projects were implemented to study experimental and mathematical predictive methods on each individual fate mechanism and to develop experimental and/or mathematical protocols where needed. Finally, a project was implemented to couple the mechanisms in a semi-deterministic predictive equation and to attempt initial verification for the equation in a continuous, complete and mixed laboratory activated sludge study.

Specific compounds studied in this project include methyl ethyl ketone, toluene, phenol, aniline, 1,4-dichlorobenzene, 2,4-dichlorophenol, and pentachlorophenol. Only certain compounds typical for the mechanism of interest were studied in each mechanism project. Both ^{14}C-labeled and nonlabeled compounds were used in the sorption, bio-oxidation, and laboratory activated sludge projects to provide independent and corroborative analysis as well as an unambiguous measure of bio-oxidation mineralization.

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FOREWORD

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water systems. Under a mandate of national environmental laws, the agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. The Clean Water Act, the Safe Drinking Water Act, and the Toxics Substances Control Act are three of the major congressional laws that provide the framework for restoring and maintaining the integrity of our Nation's water, for preserving and enhancing the water we drink, and for protecting the environment from toxic substances. These laws direct the EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The Water Engineering Research Laboratory is that component of EPA's Research and Development program concerned with preventing, treating, and managing municipal and industrial wastewater discharges; establishing practices to control and remove contaminants from drinking water and to prevent its deterioration during storage and distribution; and assessing the nature and controllability of releases of toxic substances to the air, water, and land from manufacturing processes and subsequent product uses. This publication is one of the products of that research and provides a vital communication link between the researcher and the user community.

This report presents the results of a program to develop methods for the prediction of organic chemical fates in activated sludge processes. Bio-oxidation, stripping, and biomass adsorption fate mechanisms were studied separately, and individual experimental protocols and kinetic models were developed. The resulting relationships were studied in laboratory-scale activated sludge units.

Francis T. Mayo, Director
Water Engineering Research Laboratory

ABSTRACT

This report describes results from a broadly based effort to determine the feasibility of predicting the fates of organic chemicals in diffused air activated-sludge wastewater treatment processes. The three conversion/removal mechanisms emphasized in this work are stripping, sorption on biomass, and bio-oxidation (biotransformation and mineralization). After an initial literature review and critique, separate projects were implemented to study experimental and mathematical predictive methods on each individual fate mechanism and to develop experimental and/or mathematical protocols where needed. Finally, a project was implemented to couple the mechanisms together in a semi-deterministic coupled predictive equation and to attempt initial verification for the equation in a continuous, complete-mixed laboratory activated sludge study.

Specific compounds studied in this project were: (1) for stripping - toluene, 1,4-dichlorobenzene, methyl ethyl ketone, and phenol; (2) for sorption - phenol, 1,4-dichlorobenzene, and pentachlorophenol; (3) for bio-oxidation - phenol, toluene, aniline, and 2,4-dichlorophenol; and (4) for the laboratory activated sludge study - phenol, toluene, aniline, and pentachlorophenol. Both ^{14}C-labeled and non-labeled compounds were used in the sorption, bio-oxidation, and laboratory activated sludge studies to provide independent and corroborative analysis as well as an unambiguous measure of bio-oxidation mineralization.

Stripping studies utilized a six-foot high by six-inch-diameter coarse-bubble batch reactor and included study of the effects of surfactants, salts, oils, and non-viable biomass on the stripping rate constant. An equation was developed (and verified for toluene in the laboratory activated sludge study) that relates the stripping rate constant to the ratio of air flow to liquid volume and the compound's Henry's law constant. This result, taken with work recently done by others in the field, strongly supports the hypothesis that stripping in diffused-air systems is a single-stage equilibrium process.

Work done with the contaminants described above results in changing the stripping rate constants ±50% as compared to pure water (tap or distilled) experiments. More importantly, the effect of contaminants on the stripping rate was not as pronounced as the effect on oxygen transport and predictive methods that predict stripping rates as a function of oxygen transfer for contaminated systems must be questioned.

Sorption studies used non-viable biomass of various types and a variable-volume batch reactor to determine the disappearance of various compounds due to sorption while controlling or eliminating simultaneous stripping and bio-oxidation. Correction for compounds residing in extracellular water in the biomass was also attempted. A predictive equation based on the compound octanol-water partition coefficient, the cellular lipid concentration, and the lipid density was verified for the compounds studied at the concentrations present. Verification tests in continuous lab-activated sludge studies suggest the existence of a solids saturation concentration at high mixed liquor concentrations. Sorption kinetics for the compounds studied were very fast and, except for estimation of minimum values, could not be quantified.

Bio-oxidation studies emphasized a batch mineralization assay for batch, fill-and-draw, and continuous activated sludge tests. Specific compounds and total degradative organisms were enumerated based on traditional microbiological methods. First order and second order (first order in both substrate concentration and organism counts) mineralization and disappearance kinetic rate constants were calculated. Mineralization rate constants were relatively consistent over the batch, fill-and-draw, and continuous systems. However, differences in the disappearance rate constants between both the batch and fill-and-draw reactors and the continuous LAS reactors varied by up to three orders of magnitude. Although presently unexplained, this disappearance rate variation has profound effects on the fate of a compound in a given system. Also of note is the fact that the degradative population in all reactor configurations is less than 0.1% of the total viable cells.

Laboratory activated sludge studies provided verification opportunity for each mechanism predictor and for the coupled-mechanism predictor by providing direct data on conventional and radiolabeled compound fates. These data permitted the establishment of the feasibility of these methods.

Finally, an algebraic coupled predictive equation is proposed for organic chemical fates in diffused air complete-mix activated sludge treatment processes, within the constraints of the assumptions and derivations provided. This equation incorporates stripping, sorption (below any saturation concentration), and bio-oxidation mechanisms and predicts the fractional distribution of soluble substrate fed between the above mechanism and the effluent. Upon further verification, this coupled equation may be used to quantify bio-oxidation disappearance rates in multi-mechanism tests and systems and, with reliable biological disappearance rates, may be used to quantitatively predict fates in a given system. Unverified examples of the use of the coupled predictor are presented.

This report was submitted in fulfillment of Contracts No. 68-03-3027 and 68-03-3074 by IT Corporation and the University of Tennessee (subcontract) under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period October 21, 1980 to September 30, 1984, and work was completed as of September 30, 1984.

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SECTION I

INTRODUCTION

This study was undertaken to assess the feasibility of using a mechanistic modeling approach to predict the fate of organic chemicals in an activated sludge process. The early effort on relating organic chemical structure to biological treatment dates back at least to the work of Ludzack and Ettinger presented at the 14th Annual Purdue Industrial Waste Conference, May 1959. Subsequent efforts by Chambers (1963) and Tabak (1964) provided additional insights. More recently, Cohen related adsorption of organic compounds on activated carbon and Strier developed treatment models, based on physical/chemical properties. Berkau proposed further work in this area at the 87th national meeting of the American Institute of Chemical Engineers (1979).

The basic goal of this study was to test the feasibility of application of organic chemical fate predictions to a complete mix, continuous flow activated sludge process, and to integrate into the test the existing knowledge and data on this subject. Within the limits of the funds available and the concept of a feasibility study, the project was conceptualized to include individual study of each major fate mechanism (stripping, sorption onto biomass, and biooxidation) and then integration of these results into an overall descriptive framework. Subsequent laboratory-scale testing of the combined processes was also included. A general assessment of the status and feasibility of a mechanistic approach to fate prediction in activated sludge processes is provided as a result of the determination of the strengths and limitations of this type of approach.

This report covers a very broad area, crossing many engineering and scientific disciplines. The format used attempts to segregate each section (a separate study area) and allows each to stand alone. A summary is included in each section.

Nomenclature in this report is internally consistent insofar as it is possible for a study area overlapping so many disciplines. Symbols have been assigned in general with subscripts denoting the phase or compartment on which that variable is based, and superscripts denoting the removal/conversion mechanism to which it applies. Occasionally this convention is dropped to incorporate nomenclature specifically accepted in one or another discipline represented in this report. Because of this factor and because the units of any given symbol depend on the equation in which it is used, symbols are defined as they are used. Symbol definitions either immediately precede or follow the equations in which they are used.

Most actual experimental data, the quality assurance summary, and bibliographic material are presented in the appendices to this report. The appendices are available from the EPA by special request.

BACKGROUND

Some 50,000 organic chemicals are estimated to be in commercial production in the United States, and another 500 to 600 new organic chemicals are being placed into production each year. These chemicals and their processing by-products find their way into industrial wastewaters and are often treated in biological treatment plants. Many of these organic chemicals are oxidized by biological processes, and many more can be oxidized if the treatment plant biomass can adjust or acclimate to the specific problem compound and establish a stable population. Still other organics do not undergo biological oxidation in treatment plants. The number of these compounds has been thought to be minimal, however, and until recently, their environmental and health impacts have been unknown.

Existing industrial biological treatment plants have been designed to remove specified levels of organic pollution as measured by the non-specific parameters of biochemical oxygen demand (BOD) or chemical oxygen demand (COD) to meet specified discharge criteria. Discharge criteria have often been established based on the belief that the receiving bodies possess a certain ability to assimilate BOD and that the aqueous discharge is within the assimilation capability of the natural waters. According to this theory, even difficult-to-degrade organics would eventually be converted or destroyed since the natural aquatic ecosystem is also a biological reactor, albeit one that responds more slowly than biological treatment plants.

Degradative enzymes necessary for biotransformation and bio-oxidation have generally been assumed to have co-evolved with the development of new organic molecules produced in nature. This assumption suggests that most naturally occurring organic molecules are subject to biological degradation under the proper environmental conditions. However, workers do encounter naturally occurring organic compounds such as lignin that are resistant to biological degradation (i.e., recalcitrant). Such chemicals owe their recalcitrance to specific molecular structure or polymeric assemblage of simple organic molecules into complex high-molecular-weight compounds. Recently, arguments have been posed for the existence of some synthetic organic chemicals for which no biodegradative enzymes exist. Such biorecalcitrant synthetic organics may be due to a complex polymer structure, molecular structures similar to biorecalcitrant natural molecules, or a chemical structure foreign to anything produced in nature.

Microorganisms encountering such chemicals may evolve new enzymes or develop populations able to degrade these chemicals if a selective pressure exists (i.e., available carbon or energy sources or resistance to some intrinsic toxicity of the chemical). However, even though degradation may occur, the rate of degradation may still be slow, and the chemical may accumulate in the environment faster than it can be converted to innocuous products.

The advent of analytical methods capable of detecting very low concentrations of recalcitrant chemicals has allowed scientists to track the accumulation of many organics in the environment. This fact has caused the U.S. Environmental Protection Agency (EPA) to rethink its position on standards of performance for biotreatment plants in the last few years. A greater emphasis on specific organic removal is being incorporated into treatment plant performance criteria.

Certain compounds that do not bio-oxidize have been removed from the treated wastewater in existing biological processes. In the past, any reduction in the concentration of the organic compound in the plant was attributed to the plant's biodegradative treatment efficiency. Recent investigators have tried to establish what happens to certain chemicals that are known to be incapable of bio-oxidation in treatment plants but that are nevertheless removed from the effluent. Other removal or conversion mechanisms were found to exist. When a compound has a high vapor pressure (especially with a low water solubility), much of it will be stripped from the wastewater, a process that is enhanced by the action of the plant's aerators. When a compound is very lipophilic (i.e., partitions strongly with the oil phase in an oil-water mixture), significant quantities may be sorbed on the biomass or sludge in a biological treatment plant. Certain compounds may react with chemical oxidants present, or they may be hydrolyzed or photolyzed.

The fate of a chemical may be assessed qualitatively, usually by calculations based on physical-chemical property data on its likely pathways from biological treatment plants. However, it is not possible to predict quantitatively how much of the chemical will strip, sorb, bio-oxidize, or be chemically converted when the chemical has qualities suggesting potential for more than one of the removal mechanisms. In other words, the fate of a compound that would be easily bio-oxidized but would not be expected to strip or sorb could obviously be predicted. But the fate of a compound that would bio-oxidize at a moderate rate and would be expected to strip and/or sorb would be very difficult to assess quantitatively. Measuring the specific chemical in a laboratory-, pilot-, or full-scale biological treatment plant is the only way to assess this with accuracy, and substantial concerns existed as to the applicability of these findings to other waste streams and treatment processes.

Developing a method to predict quantitatively the fates of chemicals in an activated sludge plant -- a predictive fate method (PFM) -- is a multidimensional problem. First, the theory behind the specific transport and conversion mechanisms must be known, and these mechanisms must be coupled into a combined, dynamic model. Experimental data must exist or be generated on each mechanism to evaluate and verify the model. Collection of these data must be controlled so that the effects of different mechanisms can be analyzed separately. The model or method must be extensively tested, ultimately in full-scale systems. If these conditions are satisfied, a verified PFM results.

Several additional steps are required to expand the PFM so that it may be used for chemicals on which no treatability data exist. The chemical data originally collected to develop the PFM must be examined to see if relationships exist between the treatability data and the physical, chemical, or structural properties of the chemical. These structure-treatability relationships (STR) can then be used to predict or project values for important variables appearing in the PFM. A number of additional compounds would, most likely, need to be tested to collect additional mechanism data. These compounds would be selected based on their structure or other indicative physical or chemical properties. Ultimately, a structure-treatability predictive fate method (STPFM) would be developed and verified, thereby expanding the predictive fate method to other compounds without requiring treatability studies on those compounds.

Finally, with a tool for predicting chemical fates without specific chemical treatability data, chemicals could easily be identified and grouped into categories based on their expected fates in biological treatment plants. Such a method and the resultant groupings would be scientifically based and supportable. Such methods could be used to identify problem compounds and to quantify the extent of a problem so that the regulatory need for specific standards could be determined early, thereby simplifying the development of the standard. The method could also allow the assessment of future standards for compounds not now recognized as problem compounds, thus simplifying future regulatory efforts.

Other potential benefits from verified predictive methods relate to a more detailed understanding of the basic processes at play within treatment processes. Such an understanding should lead to the definition of the limits of the treatment process and quantification of multimedia chemical transport. Possibly, insights into process improvements for existing and new plants may emerge.

This study focused on the development of mechanism predictive fate methods for an aerobic activated sludge process with diffused aeration. The aeration or basin reactor is the unit operation that is expected to be related to the majority of mechanism-related removals or conversions in an activated sludge process. Thus the mechanisms in the aeration reactor were stressed in all phases of this project.

OVERVIEW OF PRIOR WORK

A number of organizations and investigations have been actively pursuing various parts of the total problem of developing a STPFM. The EPA presented a concept of relating chemical classes or groups to treatability in 1979.[1] Although these groupings were never verified, this work suggested that if such groupings were possible, then scientifically based standards could be more efficiently and accurately developed. That concept was refined and with the following rationale led to the development of this project. If chemicals can be grouped according to treatability, the groups should be based on scientifically accepted methods for predicting common fates. That is, chemicals in similar treatability groupings should have common fates, and fate prediction should be the basis for grouping chemicals by treatability.

The vast majority of the work done on determining the fates of organic compounds has been focused on environmental fates -- that is, the fate of compounds when released into the atmosphere, surface water, ground water, or terrestrial systems. This has been important to environmental scientists because it has enabled the identification of "problem" compounds that may persist in the environment or have enhanced detrimental effects on the environment. These techniques have been developed by a host of researchers, but fortunately they have been summarized in several compendia.[2,3] In the past, the estimation methods for environmental fate analysis have focused on equilibrium conditions of specific kinetic rate processes. Often insufficient data are available on environmental systems to perform more detailed kinetic rate calculations.

When one attempts to apply some of these techniques to an operating wastewater treatment process, it becomes apparent that each removal/conversion mechanism may be intensified, and system residence times may be considerably shorter than those of natural ecosystems. For example, biomass concentrations are orders of magnitude higher than natural environmental systems, and aeration provides much higher gas-to-liquid surface contact than normal environmental situations. Subsequently, each mechanism may have different overall kinetic rates, and equilibrium in each rate process may or may not necessarily be achieved. Furthermore, the relative magnitude of rate constants between rate mechanisms may determine the dominant mechanism.

Kinetics of the stripping, sorption, and bio-oxidation mechanisms in activated sludge systems have been studied to varying extents. The literature background in each of these areas is discussed in greater detail in subsequent sections.

Considerably less work has been done on developing combined or coupled methods for determination of the overall fate of chemicals in wastewater treatment systems. The work can be grouped into efforts primarily focused on experimental determination of the fate of organics in treatment processes and efforts focused on mathematical and experimental prediction of substrate fates.

Experimental approaches include work by Kincannon, et al.[4], in which lab scale activated sludge treatment systems were operated on synthetic feeds and challenged by the addition of a variety of organic chemicals. Fates were determined by analysis of effluent and gas streams for the chemicals added. Mean percent removals were determined for each compound. Others have taken a more mathematically predictive approach by comparing experimental results in lab or pilot scale equipment with behavior predicted by theoretical kinetic equations.[5,6,7] Efforts to relate lab scale, batch or continuous findings to specific mechanism experimental methods in larger scale continuous systems are lacking. That is, the ability to predict continuous mechanism

rates and the fate of compounds from specific mechanism predictive methods has not been demonstrated.

The ability to develop estimates of mechanism kinetic rate constants from physical, chemical, or structural information on the compound of interest in treatment plants has not been fully studied. Work in the environmental fate area has led to correlations of volatilization processes with Henry's law constants,[2,3,8] sorption with octanol-water partition coefficients,[6,9,10] and bio-oxidation with octanol-water partition coefficients,[11] and other structural variations.[12,13] Excellent reviews are available covering the present state of the sorption mechanism.[14,15] Unfortunately, the majority of these studies either deal with equilibrium relationships or environmental systems with their related kinetic processes. It is unclear whether these predictors apply to the more intense conditions and rate processes of biological treatment plants.

Statistical correlations of full-scale plant data have been attempted in order to directly elucidate relationships between chemical groups and treatabilty.[16] Although important pathfinding approaches, these efforts have not been successful in developing chemical groupings that may be extrapolated beyond the compounds in the study. This is a common problem with large statistical studies because an error related to problems in the original data base is often difficult to determine and theoretical determinative understanding is often not incorporated.

PROJECT SCOPE AND OBJECTIVES

Evaluation of the literature indicated the need for a project to combine and integrate existing knowledge and to develop new experimental and mathematical protocols as necessary for predicting fates in biological treatment plants. This project was conceptualized with the following objectives.

1. To survey and assess the existing data base related to stripping, biomass sorption, and bio-oxidation mechanism rate predictions (performed in contract 68-03-3027, WA1).

2. To select a limited list of compounds for study that might indicate the behavior of many more organic compounds.

3. To develop experimental protocols to measure and quantify the kinetics of stripping, sorption onto biomass, and bio-oxidation.

4. To develop mathematical protocols or models to describe the removal/conversion rates for the stripping, sorption, and bio-oxidation mechanisms.

5. To develop an overall experimental protocol for measuring the mechanism kinetic rates in a continuous system more representative of real operating activated-sludge plants.

6. To develop an overall mathematical protocol or model to incorporate the individual mathematical predictors and describe compound fates in a complex system indicative of an operating activated-sludge plant.

7. To determine the feasibility of evaluating the kinetic rate and other constants necessary for individual or coupled mechanism fate prediction from physical, chemical, or structural properties of the compound itself.

Although it encompassed a very broad study area, the scope of the project has been controlled to limit the depth to which each of the various objectives has been investigated. Compounds have been carefully selected to be potentially representative of wider groups of organics. Project resources were controlled to avoid delving into specific research areas too deeply but to identify them nonetheless as important areas of future research.

This project was designed to answer questions at different plateaus in the overall problem of fate prediction. These plateaus fall into experimental, mathematical, and property predictive categories. Experimental concerns include adequate experimental methods for individual mechanism kinetic rate measurement and experimental determination of fates in an operating process. Mathematical concerns relate to the applicability of established kinetic models to stripping, sorption, bio-oxidation mechanisms, and coupled systems. Property predictive questions relate to the ultimate ability to arrive at fate predictions based only on a compound's physical, chemical, and/or structural properties.

Though this report does not achieve complete understanding in the deepest sense of all of these plateaus, the work performed does allow the assessment of the feasibility of success on each plateau, consistent with our original EPA charter. We hope that we have also clarified the overall field and offered some original contributions at various points.

THE ACTIVATED SLUDGE TREATMENT PLANT SYSTEM

Activated sludge treatment processes may be considered systems comprised of compartments, subcompartments, and transport/conversion rate vectors. Compartments are homogeneous phases and/or subphases. Transfer between compartments or generation or conversion of a component within a compartment is represented by a kinetic rate vector. Figure 1 presents a graphical description of the compartments and rates of the activated sludge system assumed in this project.

The activated sludge system is comprised of an aqueous, a liquid organic, a gas/vapor, and a solid compartment.

Aqueous Compartment

The aqueous compartment is the aqueous phase, absolutely free of suspended solids, undissolved liquids or undissolved gas/vapors. This is an idealized picture because much attention to sampling protocols is required in order to truly achieve aqueous samples of the aqueous compartment only. However, this more rigorous conceptual model is needed on which to develop a better understanding of inter-compartment transfer.

Liquid Organic Compartment

The liquid organic compartment is made up of two subcompartments - liquid organic substrate and other liquid organics. A system possesses a liquid organic substrate subcompartment when the substrate is an organic liquid and it is found as such in the process. Other liquid or organic phases may also be present, and hydrocarbons or other organics could be present as a separate phase. Presence of liquid organic compartments may have a profound effect upon measurement of the major fate mechanisms.

Each compartment and the system as a whole have physical state descriptors that describe the physical state of the compartments. These descriptors are important because they often affect the transport/conversion rates between and within compartments. Also, depending on the physical state descriptors, solids or liquids may be so finely divided they can leave the system with the aqueous effluent. (In this case, the effluent would contain an aqueous compartment and either liquid or solid compartments.) Liquid organics may exist as fully divided suspended phases such as emulsions or micelles or as a discrete homogeneous phase either floating on the aqueous surface or on the vessel's bottom. The degree to which the liquid organics exist in one state or the other depends upon the relative rates of dispersion and coalescence and/or settling. These are not substrate transfer processes in the same sense as transport or conversion between compartments or subcompartments, but rather describe the physical state or movement of the compartment as a whole.

The two liquid organic subcompartments would likely combine into a single organic phase if both were simultaneously present. This is shown by the mixing rate vector on the diagram. Substrate in the substrate subcompartment exists as a pure material, and transfer between this subcompartment and the aqueous compartment is either supersaturation if the concentration in the aqueous compartment exceeds the substrates' solubility or dissolution if the liquid organic phase dissolves. Partitioning of the substrate between the aqueous compartment and the liquid organic subcompartment is an extraction rate vector.

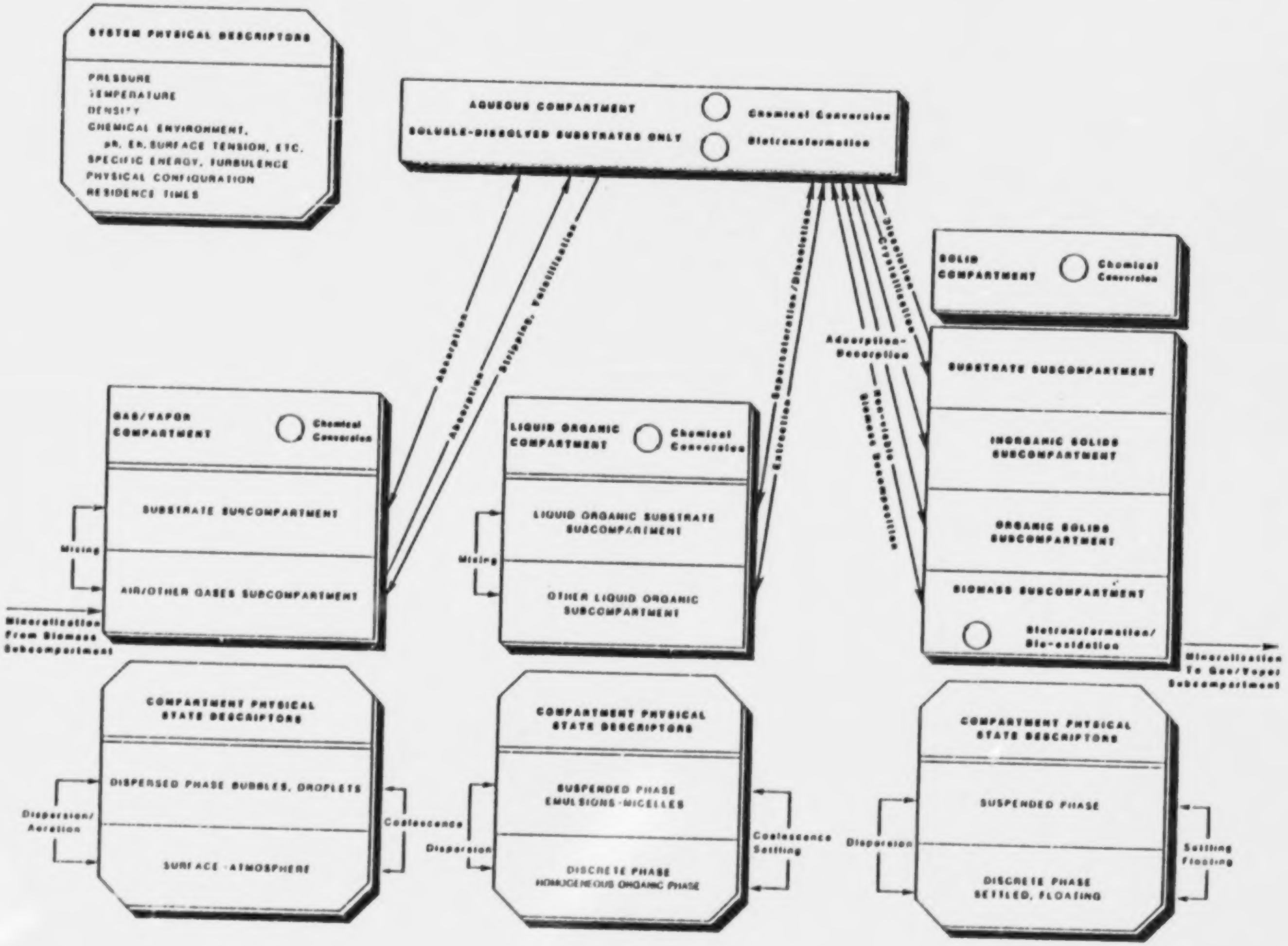
SYSTEM PHYSICAL DESCRIPTORS
PRESSURE
TEMPERATURE
DENSITY
CHEMICAL ENVIRONMENT,
pH, Eh, SURFACE TENSION, ETC.
SPECIFIC ENERGY, TURBULENCE
PHYSICAL CONFIGURATION
RESIDENCE TIMES
AQUEOUS COMPARTMENT
SOLUBLE-DISSOLVED SUBSTRATES ONLY
Chemical Conversion
Biotransformation
Absorption
Absorption
Stripping, Volatilization
Extraction
Supersaturation/Dissolution
Dissolution
Crystallization
Adsorption-Desorption
Non-viable Biomass Decomposition
GAS/VAPOR COMPARTMENT
Chemical Conversion
SUBSTRATE SUBCOMPARTMENT
AIR/OTHER GASES SUBCOMPARTMENT
Mixing
Mineralization From Biomass Subcompartment
LIQUID ORGANIC COMPARTMENT
Chemical Conversion
LIQUID ORGANIC SUBSTRATE SUBCOMPARTMENT
OTHER LIQUID ORGANIC SUBCOMPARTMENT
Mixing
SOLID COMPARTMENT
Chemical Conversion
SUBSTRATE SUBCOMPARTMENT
INORGANIC SOLIDS SUBCOMPARTMENT
ORGANIC SOLIDS SUBCOMPARTMENT
BIOMASS SUBCOMPARTMENT
Biotransformation/ Bio-oxidation
Mineralization To Gas/Vapor Subcompartment
COMPARTMENT PHYSICAL STATE DESCRIPTORS
DISPERSED PHASE BUBBLES, DROPLETS
SURFACE-ATMOSPHERE
Dispersion/ Aeration
Coalescence
Dispersion
COMPARTMENT PHYSICAL STATE DESCRIPTORS
SUSPENDED PHASE EMULSIONS-MICELLES
DISCRETE PHASE HOMOGENEOUS ORGANIC PHASE
Coalescence Settling
Dispersion
COMPARTMENT PHYSICAL STATE DESCRIPTORS
SUSPENDED PHASE
DISCRETE PHASE SETTLED, FLOATING
Settling Floating

FIG. 1. COMPARTMENTS IN AN ACTIVATED SLUDGE SYSTEM

Gas/Vapor Compartment

The substrate may also transfer from the aqueous compartment into the gas/vapor compartment. Rarely, the organic substrate may exist as a gas or vapor (ethylene, for example), in which case, it is represented by the substrate subcompartment. In most cases, the gases or vapors present are air, oxygen, or other gases produced by reaction or evolved from solution. The physical state descriptors range from a completely dispersed phase such as bubbles in liquid or droplets in air to a continuous surface such as the atmospheric interface. Aeration processes with either diffusers or surface aerators generate dispersed phases with high interfacial areas, while coalescence leads to continuous surfaces with minimal interfacial area. Again, the physical state descriptor often effects the transport/conversion rate vectors through modification of the interfacial area.

Transport of substrate between the gas/vapor substrate subcompartment and the aqueous compartment is an absorption rate vector. Transport between the aqueous compartment and the air/or the gas subcompartment is either by stripping or volatilization. In this report, stripping is defined as the interfacial transport process when a dispersed phase is present. Volatilization represents the transport process when the gas-liquid interface is a continuous surface. Stripping predominates in activated sludge treatment processes because of the high fraction of interfacial area related to the existence of a dispersed phase.

Solid Compartment

The solid compartment is the most complex of the compartments. It contains the substrate subcompartment when the substrate is normally a solid and is found as an undissolved solid in the process, an inorganic solids subcompartment, an organic solids subcompartment, and a biomass subcompartment. The substrate subcompartment interacts with the aqueous compartment through crystallization-dissolution rate transport vectors, depending on the aqueous phase concentration vs. the substrate aqueous solubility. Transport occurs between the other solid subcompartments related to physical or chemical sorption and desorption rate vectors.

The biomass subcompartment may be further subdivided into other subsections distinguishing between nonviable biomass, viable biomass that degrades or biotransforms the substrate, and viable biomass that utilizes organics other than the substrate in the aqueous compartment. Nonviable biomass undergoing decomposition potentially releases substrate (and other organics) back to the aqueous compartment. Solids in the system may be present as suspended phases or discrete (settled or floating) phases, depending on the relative dispersion.

It is important to note that the above system may only be rigorously determined when the concentrations and subcompartment of substrate in each compartment can be determined, the importance of each rate vector is known, and each important vector is quantified. Past work in the determination of fates in lab-, pilot-, and full-scale activated sludge systems has been limited in the ability to distinguish among various subcompartments of the nonsoluble phase. Mixed liquor volatile suspended solids (MLVSS) have traditionally been used as an indicator of the concentration of biological solids. However, MLVSS determinations also include any volatile nonbiological solids in the influent wastewater. Determination of sorption of organic substrates to biological solids has also been hindered by the limited ability to distinguish between various phases in sludge samples. In truth, sampling and analytical methods to distinguish between those compartments and subcompartments and to subsequently develop understanding of rate vectors is quite difficult and promises to be a major barrier limiting a mechanistic approach.

The system studied in this project (Fig. 2) was a much simplified version of the one presented in Fig. 1. Only a few of the above mentioned compartments and rate vectors existed in systems studied in this project.

Stripping was studied in individual experiments and in continuous, completely mixed laboratory-scale activated sludge (LAS) systems. Sorption was quantified in a specially designed variable-volume reactor and in most LAS experiments. Desorption was not quantified but was doubtlessly present in our experiments. Bio-oxidation was studied both in terms of primary degradation (substrate disappearance, biotransformation) and mineralization. Releases of substrate and other organics related to decomposition of nonviable biomass were also present in our experiments, though only estimated.

The use of a biomass culture which had been acclimated to the test substrate (Section 5) and careful substrate and nutrient addition eliminated significant amounts of inorganic solids, organic solids, substrate solids and oils, and other liquid organic oils from the study. None of the substrates studied was a vapor or gas at normal conditions. Other than ionization-conversion reactions, chemical conversion of the compounds is unlikely at the conditions studied.

Relationship of Compartments to Process Streams

Definition of the system in terms of compartments is somewhat different than using more conventional designations of process streams. To dispel possible confusion, this section will discuss that relationship. The compartmentalized system as presented in Fig. 1 is based on the possible discrete phases where a substrate may reside and the transport/conversion mechanisms that may cause compartment transfer and conversion. These homogeneous phases are compartments and may be present in any or all of the process streams in an activated sludge process similar to the one shown in Fig. 3.

For instance, process streams such as the secondary influent, mixed liquor, secondary clarifier effluent, return sludge, and others may have all of the compartments and subcompartments present, although to a much different degree. Depending on the system and compartment physical state

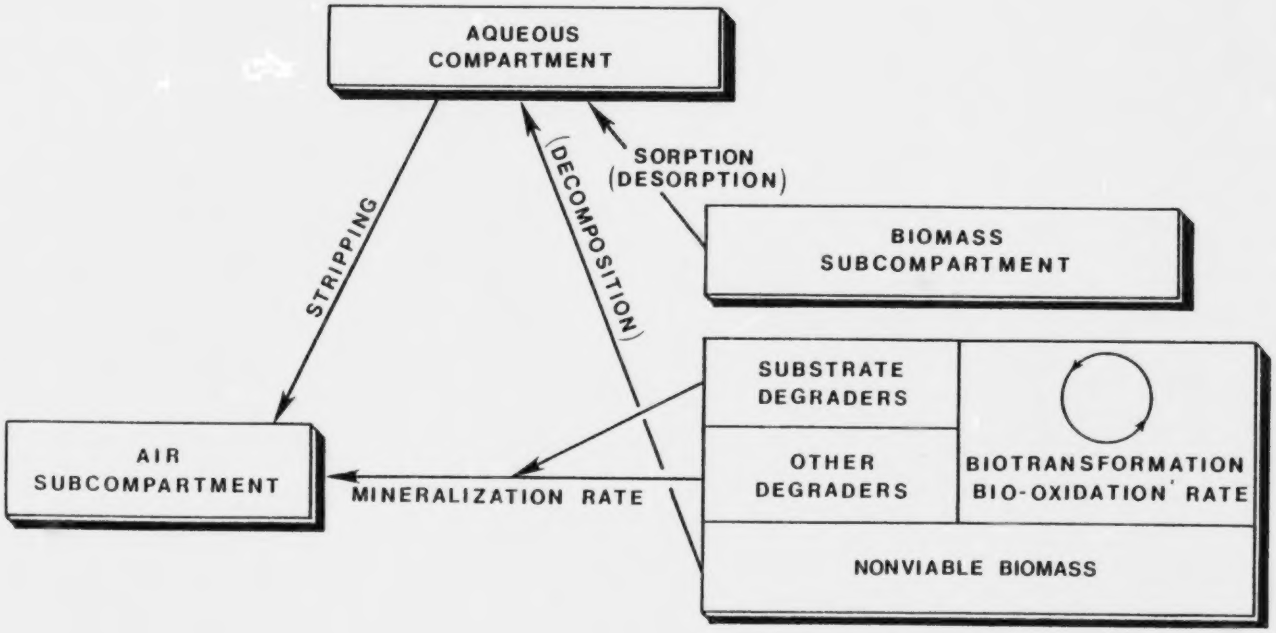
AQUEOUS COMPARTMENT
SORPTION (DESORPTION)
BIOMASS SUBCOMPARTMENT
STRIPPING
(DECOMPOSITION)
SUBSTRATE DEGRADERS
OTHER DEGRADERS
BIOTRANSFORMATION BIO-OXIDATION RATE
NONVIABLE BIOMASS
AIR SUBCOMPARTMENT
MINERALIZATION RATE

FIG. 2 SIMPLIFIED SYSTEM STUDIED IN THIS PROJECT

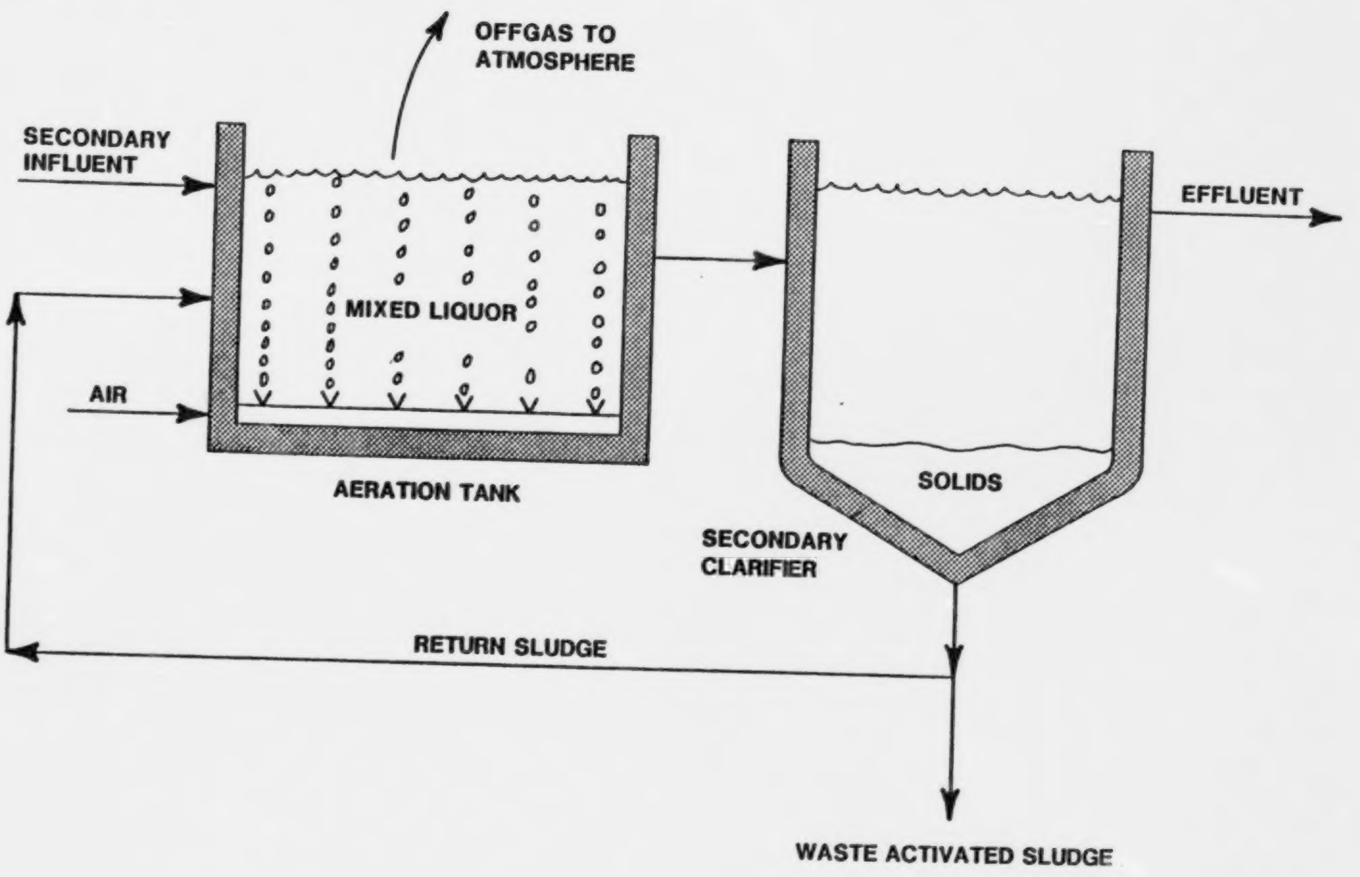
OFFGAS TO
ATMOSPHERE
SECONDARY
INFLUENT
MIXED LIQUOR
AIR
AERATION TANK
EFFLUENT
SOLIDS
SECONDARY
CLARIFIER
RETURN SLUDGE
WASTE ACTIVATED SLUDGE

FIG. 3. MODEL ACTIVATED SLUDGE PROCESS

descriptors (and the rate vectors), the final effluent may consist of only an aqueous compartment or may have substantial levels of liquid organic and/or solid compartments. In essence, the level of performance of a process may be determined by the presence or absence of a compartment in a process stream as well as the concentration of the substrate in each compartment. Thus, performance parameters such as effluent suspended solids indicate the presence of several liquid organic or solid compartments, all lumped together in one parameter.

TRANSPORT/CONVERSION MECHANISM RATE EQUATIONS

A general kinetic mathematical expression describing the change of substrate concentration in the aqueous compartment at any time is as follows:

$$r^{tot} = \frac{dC_a}{dt} = r^a + r^s + r^h + r^b + r^o + r^p \qquad \text{Eqn. 1}$$

where:

r^{tot} = the overall rate or rate equation describing substrate concentration changes related to all mechanisms; dC_a/dt is the instantaneous rate of change of the substrate concentration in the aqueous compartment (mg L^{-1} hr^{-1}),

r^a = the net rate or rate equation describing substrate concentration changes related to sorption-desorption processes in the aqueous compartment (mg L^{-1} hr^{-1}),

r^s = the net rate or rate equation describing substrate concentration changes related to stripping, volatilization, or absorption processes in the aqueous compartment (mg L^{-1} hr^{-1}),

r^h = the rate or rate equation describing substrate concentration changes related to hydrolysis chemical conversion processes in the aqueous compartment (mg L^{-1} hr^{-1}),

r^b = the net rate or rate equation describing substrate concentration changes related to biotransformation--bio-oxidation processes in the aqueous compartment (mg L^{-1} hr^{-1}),

r^o = the rate or rate equation describing substrate concentration changes related to chemical oxidation conversion processes in the aqueous compartment (mg L^{-1} hr^{-1}), and

r^p = the rate or rate equation describing substrate concentration changes related to chemical photolysis in the aqueous compartment (mg $L^{-1}hr^{-1}$).

The convention adopted in this report is that rate processes generating substrate in the aqueous compartment will be shown as positive and rate processes depleting substrate will be shown as negative. This convention is consistent with chemical engineering reactor design approaches.

Since few, if any, chemical conversion processes other than ionization are expected for the compounds and conditions studied, this relationship simplifies to:

$$r^{tot} = \frac{dC_a}{dt} = r^a + r^s + r^b \qquad \text{Eqn. 2}$$

The net rate equation for sorption is the difference between the desorption and sorption rates or:

$$r^a = r^d - r^{sp} \qquad \text{Eqn. 3}$$

where:

r^a = the net rate or rate equation describing substrate concentration changes related to sorption-desorption processes in the aqueous compartment (mg L^{-1} hr^{-1}),

r^d = the desorption rate or rate equation (mg L^{-1} hr^{-1}), and

r^{sp} = the sorption rate or rate equation (mg L^{-1} hr^{-1}).

One hypothesis that is tested in this work is that sorption processes in biomass are actually partitioning of the substrate between the lipophilic fraction of the biomass and water. Assuming that biological lipids have the same density as n-octanol, the following rate equation is postulated for sorption onto biomass from the aqueous compartment.

$$-r^{sp} = -\frac{dC_a^{sp}}{dt} = K_a^{sp}\left[\frac{f_L K_{ow} C_a}{\rho_L} - C_{sb}\right] \qquad \text{Eqn. 4}$$

where:

$-\frac{dC_a^{sp}}{dt}$ = the instantaneous rate of decrease of substrate concentration in the aqueous compartment related only to biomass sorption processes (mg L^{-1} hr^{-1}),

K_a^{sp} = the substrate biomass sorption rate constant based on aqueous compartment disappearance (hr^{-1}),

X = the concentration of biomass in the system (mg biological solids/L),

f_L = the lipid mass fraction of the biomass (mg lipids/mg biological solids),

K_{ow} = the octanol-water partition coefficient (unitless concentration ratio),

C_a = the substrate concentration in the aqueous compartment (mg/L),

ρ_L = the density of the lipids (mg/L), and

C_{sb} = the equilibrium substrate mass loading in the biomass solids subcompartment (mg substrate/mg biological solids).

This study did not investigate desorption rates of substrate from biomass. This process and its rate equation are discussed more fully in Sections 8, 10, and 11.

The net rate of substrate concentration change related to stripping, volatilization, and absorption processes, r^s, has the form of:

$$r^s = r^{ab} - r^{st} - r^v \qquad \text{Eqn. 5}$$

where:

r^{ab} = the rate equation for absorption of substrate from the gas-vapor compartment into the aqueous compartment,

$-r^{st}$ = the rate equation for stripping of substrate from the aqueous compartment into the gas-vapor compartment, and

$-r^v$ = the rate equation for volatilization of substrate from the aqueous compartment into the gas-vapor compartment.

Again, the distinction between stripping and volatilization is that stripping occurs between dispersed gas-liquid phases (bubbles, droplets) while volatilization occurs at the gas-liquid surface.

In activated sludge processes, r^{st} is assumed to be much more significant than r^v because the gas-liquid interfacial area in dispersed phase systems is much greater than the continuous liquid surface and r^{ab} is assumed negligible since the concentration of substrate in the feed air is rarely significant. Therefore, in this study, $r^s = r^{st}$, and r^{st} may be described as follows:

$$-r^{st} = -\frac{dC_a^{st}}{dt} = K_a^{st} a\, C_a \qquad \text{Eqn. 6}$$

where:

$-\frac{dC_a^{st}}{dt}$ = the instantaneous rate of decrease of substrate concentration in the aqueous compartment related only to stripping of substrate into the gas-vapor compartment (mg L^{-1} hr^{-1}), and

$K_a^{st}a$ = the substrate stripping rate constant based on aqueous phase disappearance (hr^{-1}).

Relationships between $K_a^{st}a$ and oxygen transfer rate coefficients, K_La, oxygen and substrate diffusivity ratios, and liquid and gas mass transfer film coefficients have been proposed. This study proposes a direct relationship between $K_a^{st}a$ and the substrate Henry's law constant for activated sludge processes. These relationships are discussed further in Section 6.

The bio-oxidation - bio-transformation rate equation, r^b, can be written as a first order rate equation, an overall second order rate equation (first order in substrate concentration and first order in biomass concentration), and, using Michaelis-Menten and Monod kinetics, as a zero-to-first order equation depending on substrate concentration and the substrate half-saturation constant.

The first and second order equations are as follows:

$$-r^b = -\frac{dC_a^b}{dt} = K_1^b C_a \qquad \text{Eqn. 7}$$

$$-r^b = -\frac{dC_a^b}{dt} = K_2^b C_a X \qquad \text{Eqn. 8}$$

where:

$-\frac{dC_a^b}{dt}$ = the instantaneous rate of decrease in concentration the aqueous compartment related only to bio-transformation of substrate into other compounds,

K_1^b = the first order substrate bio-transformation rate constant based on aqueous phase disappearance of substrate (hr^{-1}),

K_2^b = the second order substrate bio-transformation, rate constant based on aqueous phase disappearance of substrate ($L\ hr^{-1}$ biomass $unit^{-1}$), and

X = the concentration of biomass in the system (biomass unit L^{-1}).

The above rate equations are for substrate disappearance. Similar equations may be written based on the rates of substrate converted to CO_2. This is called the mineralization rate and will be denoted with the additional superscript, m. Therefore:

$$-r^{bm} = -\frac{dC_a^{bm}}{dt} = K_1^{bm} C_a \qquad \text{Eqn. 9}$$

$$-r^{bm} = -\frac{dC_a^{bm}}{dt} = K_2^{bm} C_a X \qquad \text{Eqn. 10}$$

The Monod modification of Michaelis-Menton kinetics incorporates the fact that bio-transformation kinetics are enzyme-mediated and enzymes are catalysts.

$$-r^{b} = -\frac{dC_a^{b}}{dt} = \frac{1}{Y}\,\mu_m\,\frac{C_a X}{K_s + C_a} \qquad \text{Eqn. 11}$$

where:

Y = the yield coefficient and is equal to $\frac{dX}{dC_a}$ (mg biomass/mg substrate removed),

μ_m = the maximum specific substrate utilization rate when $C_a >> K_s$ (hr^{-1}), and

K_s = the saturation constant and is equal to the substrate concentration at the growth conditions when the specific growth rate is equal to $\mu_m/2$ (mg substrate/L).

These and other biological rate equations are discussed further in Sections 9 and 10.

OVERALL DESIGN EQUATIONS

Rate equations may be used to determine the overall conversion or generation of substrates in the activated sludge system depending on the specific design of the biological reactor. Complete treatments of this area have been presented. This section is based on a presentation taken from Levenspiel.[17] It is assumed here that the bio-reactor of an activated sludge system is the aeration basin or tank, although it should be noted that various transport/conversion mechanisms can occur in other system unit operations.

Activated sludge systems may be designed as batch or continuous systems. Batch systems are rarely used in full scale designs, but are often used in bench- and pilot-scale microbiological and engineering experimental studies. Full-scale fill-and-draw or sequencing batch reactors have been proposed and used for some waste treatment applications. Continuous systems may be completely mixed or plug flow in design. Completely mixed processes have the same substrate concentration everywhere in the vessel, while plug flow systems have a relatively high substrate concentration in the initial lengths of the reactor and diminish (usually nonlinearly) as the fluids proceed down the plug flow reactor. Although it is possible to closely approximate ideal complete-mixed or plug flow designs at small scales, most full-scale plants are neither ideal complete-mixed or plug flow designs. Experimental tracer methods exist to quantify how closely any given design approximates either ideal design, and these methods are described in Section 6 and other references.[17]

The design equations for system performance for batch, plug flow, and completely mixed reactors for a fluid that does not change density are given below:

Batch

$$BHRT = C_{ao} \int \frac{dREM_a}{r_{tot}} \qquad \text{Eqn. 12}$$

Plug Flow

$$HRT = C_{ao} \int \frac{dREM_a}{r_{tot}} \qquad \text{Eqn. 13}$$

Completely Mixed

$$HRT = C_{ao} \frac{REM_a}{r_{tot}} \qquad \text{Eqn. 14}$$

where:

BHRT and HRT = the hydraulic residence time for the batch and continuous systems, respectively, (hr),

C_{ao} = the initial or feed concentration of substrates in the aqueous compartment (mg L^{-1}),

REM_a = the removal or conversion of the substrate from the aqueous compartment (dimensionless), and

r_{tot} = the overall applicable rate or rate equation (mg L^{-1} hr^{-1}).

It is noted that from a design or performance standpoint, plug flow and batch reactors are identical. The equations relating system parameters to substrate conversion are the same.

In addition, it is noted that the performance of both batch and plug flow systems contains the integral of instantaneous substrate conversion or $dREM_a$. Conversion may be stated in terms of concentration changes (in a constant volume system). Therefore, $REM_a = (C_{ao} - C_a)/C_{ao}$. Also, r_{tot} is a function of C_a except for zero order rates. In the cases of more than one major mechanism, the integral becomes difficult to analytically evaluate and the time- or length-based substrate concentration profile is often required to solve the relationship. The mathematics describing batch or plug flow systems inherently require experimental substrate-concentration vs. time data for multi-mechanism systems while the complete-mixed design only requires information on C_a, since C_a is uniform throughout the reactor.

Finally, it may be shown that from a mathematical standpoint, the performance or conversion of an infinite number of identical volume, complete-mixed reactors in series is exactly the same as for a plug flow reactor. That is, if one were to have an infinite number of identical, complete-mixed reactors in series all totaling the same volume as a plug flow reactor, the conversion in the complete-mixed reactors would be identical with the plug flow. In fact, at a substrate conversion level of 99% (assuming first order rates), two complete-mixed reactors in series having about four times the volume of a plug flow reactor will provide identical conversion. As the number of complete-mixed reactors increases, the total volume of complete-mixed reactors, for the same conversion, approaches the volume of the plug flow reactor. Similar relationships apply for rate equations of various orders.

Microbial populations and specific substrate degradation kinetics are functions of the organic composition and concentrations in the aqueous compartment as well as other factors, and the biomass characteristics would change in a batch or plug flow reactor system. The biomass at the end of the reactor or reaction sequence would use different substrates since much of the relatively degradable organic substrate would have been used in the initial reaction. This effect may require testing of several complete-mixed reactors in series in order to better incorporate these biomass effects.

These relationships offer hope that if data are collected on a well-designed, complete-mixed system (possibly several reactors in series), the rates determined can be extrapolated to other designs (batch and plug flow). Also, it appears feasible to analyze the performance of full-scale plug flow plants through mathematical analogy to a finite number of completely mixed sections in the plant. Sampling and analytical data on plug flow plants should possess as many equally spaced sampling points along the length of the reactor as the number of equivalent complete-mixed reactors to be assumed (from 2 to 10 reactors should achieve an approximation of sufficient accuracy). Further discussion of these general relationships as applied to wastewater treatment processes is presented in Sections 9 and 10.

PROBLEMS AND LIMITATIONS

Although the definition of a compartmental system and rate vectors offers a way to quantitatively define the fates of organics in activated sludge processes, a number of problems and limitations exist today that may preclude immediate application of this approach, at least for some compounds and some systems. However, we believe that most of these issues will yield under further study. This section discusses some of the problems requiring further effort.

The first limitation relates to the broad but shallow scope of this work. Although successful experimental protocols were developed in stripping, sorption, bio-oxidation, and lab activated sludge areas, limitations on the number of compounds to be studied required us to qualify all of our conclusions as "tentative." More compounds in each of the areas discussed above need to be studied before results can be applied to other compounds with confidence.

In the stripping area, experimental and mathematical findings appear to be successful and widely applicable to other compounds and various system designs. In the sorption area, prediction of equilibria based on lipid content and compound properties appears hopeful, but procedures for deriving K_{ow} values for real world matrices (not pure water and octanol) and measuring lipid content in biomass are lacking. These may be needed for resolving some of the variances seen in our data. Furthermore, it appears that our experimental procedures and equilibrium equations may require modification for application to higher substrate concentrations for high K_{ow} compounds. A saturation effect appears to exist at higher substrate concentrations. Presently, the saturation effect must be determined experimentally.

Bio-oxidation studies have established the utility of rate analysis incorporating the active degrading population. We are optimistic that this type of data may offer the basis for future prediction of bio-oxidation rates from chemical structure considerations. However, measurement of the degradative population by classical microbiological techniques for difficult-to-oxidize compounds (dichlorophenol and dichlorobenzene) was not effective. New methods are needed to measure the degradative populations of slowly degrading compounds. DNA probe techniques appear especially promising.

Biological compound disappearance rate data from batch or fill-and-draw reactors were not consistent with those determined in the continuous LAS reaction. This effect can also be seen in data from other investigators. This raises questions as to the validity of using any unsteady-state test results (batch or fill-and-draw) for the estimation or prediction of specific compound disappearance in continuous flow treatment plants. Kinetic substrate disappearance rate constants determined in batch systems must employ a wide range of initial substrate concentrations. Evidence is presented in this study that high initial substrate concentrations in batch tests may inhibit biological oxidation and lead to rate constants significantly lower than those measured in continuous systems. Also, the dynamics of substrate, nutrient, and/or biomass changes in unsteady-state tests may disrupt the biological activity being measured and result in rate estimates several orders of magnitude different than those experienced in continuous systems. This also raises questions about the effects of transients in continuous systems on rates and performance. At this time, neither an experimental nor a mathematical bio-oxidation substrate conversion rate predictor exists.

Because of the uncertainty in the bio-oxidation and sorption expressions, an overall mathematical PFM is only proposed (Section 11). However, an expression testing the conversion of a volatile chemical (toluene) has been verified, and an expression dealing solely with sorption has been tested (Section 10). No compound studied in the LAS runs would allow simultaneous testing of all three mechanisms.

The system studied in this project was simplified and idealized. It did not contain many of the subcompartments or rate processes that may be found in real plants. Successful experimental and mathematical protocols from this work can only be used as an idealized yardstick with which to compare full-scale processes. Major departures from the ideal case (this work) would suggest other mechanisms at play, other subcompartments present, or major dynamic transient effects occurring in a full-scale plant.

One such departure from this study is the definition of sludge or biomass in other work, including large plant sampling campaigns. When determining either the sorption rate or biological rate using techniques described in this work, one must assume that the amount of viable, nonviable, and degradative biomass is known. Sludge measurements in other studies have almost exclusively been based on gravimetric determinations (MLSS, MLVSS) that do not distinguish between organic liquids, inorganic solids, organic solids, and biological solids. Therefore, if, say, sorption onto inorganics or dissolution of solid substrate is present and causes significant effects, then major departures could occur from the ideal case studied in this work. Work is needed to assess the importance of these additional compartments and subcompartments and determine analytical methods for first measuring the important ones and then measuring the substrate concentrations in full-scale plants.

Of special concern is the variability of the amount of water in sludge samples. Biomass itself contains a high percentage of water, but it is well known that activated sludges tend to hold major quantities of extracellular water in their matrices. Measurements of compounds sorbed on biomass should include the substrate in the cell but not that contained in extracellular water. The amount of substrate present in extracellular water may be very large compared to the amount truly in or on the cell, especially for lipophobic compounds. This is clearly a function of how the sample was taken and prepared (neat, settled, filtered, or centrifuged). Studies where sludge samples are taken and extracted for analysis with no attempt made to estimate the amount of substrate in extracellular water lead to incomparable results. This study attempts to correct for this effect, but comparison of these results with other studies must be made cautiously.

With these limitations and with further work to resolve major problems, it is believed that development of experimental protocols for rate and compartment measurements are feasible. Mathematical relationships known or proposed today are expected to be verifiable with improved experimental techniques, leading to quantitative fate determination. Prediction of fates from physical, chemical, or structural properties tentatively exists now for stripping (based on the Henry's law constant) and should be possible for sorption with further work (based on K_{ow}, lipid content, real waste correction factors, and saturation limits). Resolution of a test technique suitable for collection of scalable biological rate data and procedures that allow the quantification of the degradative population in mixed cultures should yield data that can be tested for structure-treatability relationships. Although such analysis is possible only for a handful of compounds today, we believe that understanding of such predictions will increase as data become available.

FEASIBILITY OF FATE PREDICTORS FOR ORGANICS IN ACTIVATED SLUDGE SYSTEMS

A basic goal of this project was to integrate existing knowledge and data relating to organic fate prediction and test the feasibility of application to complete-mix, continuous flow-activated sludge processes. The effort considered three separate approaches to fate prediction. The first approach relates to predictions based on actual fate data and related experimental methods. Fates may be determined for each system of importance if adequate experimental methodology exists.

The second approach uses data on specific compounds and deterministic understanding of the fate mechanisms to predict fates for different compounds and systems.

The last approach uses deterministic understanding coupled with data on physical, chemical, or structural properties of the specific chemicals to predict fates of different chemicals from property data alone.

Our estimation of the feasibility of each of these approaches for the individual mechanisms is summarized in Table 1. Stripping measurement by sampling and analysis is presently feasible. It should be noted that gas analysis is more reliable for determining stripping rates of compounds with low Henry's law constants while liquid analysis is more reliable for compounds with high Henry's law constants. Stripping prediction with deterministic predictors is feasible if the stripping predictor presented in this work is validated on more compounds. Further studies on contamination effects are also needed for more compounds and system configurations. This prediction is also based on physical properties of the substrate. Therefore, predictions based on substrate properties are also feasible with further work.

We are optimistic that sorption experimental data determination is feasible with the methods employed in this project. This needs to be confirmed for more compounds over a wider range of concentration. Modifications to include experimental determination of the saturation effect are required. Similarly, we are optimistic that these data may be applied using deterministic predictors. Work targeted at predictions of the saturation concentration is required. Again, the prediction is based on physical properties and measured levels of lipids. If a satisfactory property-based prediction of saturation concentration becomes evident, then physical-, chemical-, or structural-based predictions may be feasible.

TABLE 1. SUMMARY OF THE FEASIBILITY OF ORGANIC FATE PREDICTIONS FOR ACTIVATED SLUDGE PROCESSES

Application	Determination by experimental data	Deterministic predictors based on experimental data	Deterministic predictors based on substrate properties
Stripping	Feasible at present	Feasible with further work	Feasible with further work
Sorption	Probable with further work	Probable with further work	Probable with further work
Bio-oxidation	Possible with further work	Possible with further work	Inconclusive-further work needed
All three mechanisms-			
Continuous activated sludge systems			
Lab scale	Feasible at present[a]	Possible with further work	Inconclusive-further work needed
Full scale	Probable with further work[b]	Possible with further work	Inconclusive-further work needed

[a] Assuming an experimental system is controlled to minimize compartments.

[b] Assuming that complete compartment sampling and analysis are not needed. Some compartments and rates are not significant.

Present experimental approaches, with modification, appear to be capable of determination of bio-oxidation mineralization rate data. Modifications include time-course experiments designed to yield data for determination of the order of the rate equation and improved methods for determining viable degrader populations in mixed cultures. These experiments must be conducted over a broad range of concentrations to examine concentration dependence of the rates observed.

Substrate bio-transformation (compound disappearance) rates measured in batch systems appear to be substantially lower than rates measured in continuous systems. This needs further investigation since specific compound disappearance rates are required for quantitative fate prediction. The deterministic understanding of bio-oxidation rates is sufficient so that when the experimental determination of disappearance rates and the quantitative determination of degrader populations becomes feasible, the deterministic prediction may also be possible. The prediction of bio-oxidation rates from substrate property data may be possible, but no suitable data on substantially different types of compounds were collected. As more experimental data are collected on different compounds, the ability to assess the feasibility of substrate property-based bio-oxidation prediction will be enhanced.

Collection of combined experimental data on continuous systems is feasible in controlled lab experiments, but may be difficult in full-scale systems if many compartments and subcompartments are present and if other transport/conversion rates are important. Studies related to sampling and analysis of substrates in subcompartments and the importance of hitherto unstudied rate mechanisms are needed. Experimentally based predictions should be possible as the problems noted above with the individual mechanisms are cleared up. Complete fate prediction based on compound properties for continuous systems depends on resolution of problems in sorption and bio-oxidation property-based predictors.

If the property prediction of bio-oxidation rates proves elusive, then methods on stripping and sorption may still be used to correct experimental data, allowing determination of corrected bio-oxidation rates. This may still lead to methodology allowing estimation of fates in full-scale treatment plants within a certain error factor.

The authors are optimistic that with continued efforts in this area, the "art" of biological treatment plant design may become more of a science. Basic limitations of the process for specific compound removal, an understanding of multimedia environmental effects, and specific plant design improvements may be elucidated.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

This section includes the major conclusions and recommendations from this work. Further discussions of these conclusions and recommendations and presentation of more detailed conclusions are generally given in each section.

1. This report meets a need in the area of fate prediction of organic chemicals in wastewater treatment processes. It represents a broad overview assessing the feasibility of using both experimental and mathematical predictors to quantitatively determine fates.

2. Three levels of fate prediction exist. These are prediction directly with experimental data (by directly measuring the fates), prediction using deterministic equations supplemented with experimental data, and prediction using deterministic (or stochastic) equations using only physical-chemical properties of the substrate. Each of these levels can be applied to the individual fate mechanisms (stripping, sorption, and bio-oxidation) and to coupled systems with all of the fate mechanisms. Feasibility therefore must be determined for twelve separate cases.

Generally, the feasibility of experimental fate predictions in small-scale systems can be accomplished or can be developed for all individual or coupled mechanisms. However, experimentally determining fates in full-scale systems is less assured and further development may be required.

The feasibility of fate predictions using deterministic equations coupled with experimental data is also likely, with additional development and verification needed for all of the mechanisms (especially sorption, bio-oxidation, and the coupled mechanisms).

Prediction with deterministic equations using substrate properties or structure-treatability relationships appears hopeful for stripping and sorption. However, little or no kinetic bio-oxidation data exist where both the competitive mechanisms are controlled and the reactor-design constraints are quantified. Therefore, determination of the feasibility of structure-treatability based deterministic predictors must await the generation of a suitable data base.

3. Methodology using chemical engineering kinetic approaches and reactor-design equations appears to be a viable way to analyze multi-mechanism processes for various reactor configurations.

4. The number of transport and conversion mechanisms potentially present in real full-scale plants exceeds the present capability for analysis and individual mechanism quantification. Therefore, after verification the more simplified coupled predictive methods should be viewed as useful idealized "yardsticks" against which full-scale plant performance can be compared. Work to determine the importance of the other mechanisms and processes should be undertaken.

5. The data base existing at the beginning of this effort was limited because of the specific emphasis given to different questions in each study and to the past focus on removal as opposed to specific chemical fates. Some indications on relative fate processes may still be drawn, particularly from more recent studies that look at numerous compounds with the same experimental protocol. Many of these studies are invaluable in highlighting methods and problems to be overcome in this and other future work.

6. Compounds were selected to be representative for a wide range in expected stripping, sorption, and bio-oxidation activity. Though successfully representing broad categories of organic compounds, further work with more compounds is needed to establish the universe of organic chemicals to which each of the predictive methods applies. A subset of chemicals that represents specific exceptions to the predictive methods are to be expected and these limitations can only be established with well-designed experimental programs.

7. At the request of EPA, most experimental work was done on a real waste by challenging it with the chemicals of choice. This approach provided some interesting results that may not have been as apparent if a defined media had been synthetically prepared. However, the logistics of locating a suitable waste stream and shipping and storing large volumes of it are formidable. This requirement should only be imposed on new work when issues of stream-to-stream variation emerge and as the general understanding of the mechanisms and methodologies improves.

8. Stripping in complete-mixed, diffused air systems has been found to be essentially an equilibrium process, and predictive equations are proposed. Verification on other compounds and system configurations is suggested.

9. The effects of contaminants (surfactants, salts, oils, and nonviable biomass) are significant on oxygen transfer rate constants and less significant on stripping transfer processes. Stripping rate estimates based on oxygen transfer rates for contaminated waters such as treatment plant mixed liquors must be questioned.

10. Experimental determination of stripping rates for compounds with low Henry's law constants is more easily accomplished by quantification of the

vapor stream while higher Henry's law compounds can be more reliably determined by measuring changing aqueous phase concentrations. Both air and aqueous phases should be quantified in order to check material balances and to indicate other potential removal mechanisms (i.e., chemical hydrolysis).

11. The engineering configurations and behavior of all small-scale equipment setups should be measured and reported. This includes the extent of mixing and dispersion in the equipment.

12. The effects of biomass without the bio-oxidation mechanisms were tested in stripping and sorption experiments. Various methods of preparation of nonviable biomass were tested with the selection of a process where the biomass is lyophilized and then heated at 105°C for three hours.

This process may, however, change the chemical composition sufficiently to bias the results, particularly in sorption. Further studies of this methodology are needed that quantify chemical (particularly lipid) concentration charges in the non-viable biomass. Change in morphological characteristics should also be emphasized.

13. Sorption was quantified in a variable-volume batch reactor using non-viable biomass and later in continuous lab activated sludge studies. An equilibrium deterministic predictive equation is proposed for low aqueous concentrations. Kinetic sorption rates for the compounds studied could not be quantified because of their rapidity compared with the time required for sampling and analysis. This predictive approach did not appear to apply to aniline in LAS work and only to pentachlorophenol assuming partial ionization and low concentrations. The method appears useful for other compounds and an understanding of its applicability should be developed in further work with other compounds.

14. Data on pentachlorophenol in the sorption experiment and in the LAS work suggest a saturation value for biomass sorption of about 2 μg PCP/g dry biomass. These data fit the Langmuir isotherm and if corroborated in further work could extend the ability to predict biomass sorption to concentrations of interest in industrial wastewater and hazardous waste impoundment leachates. This area deserves further emphasis.

15. Numerous kinetic rate constants for biological processes exist and can be formulated. Each of these rates applies to specific cases in substrate and biomass concentration and reactor configuration. Little reliable data exist in this area, and accepted overall methods are lacking.

16. First and second order (first order in both substrate and biomass concentration) rate constants are developed in this study for batch, fill-and-draw, and continuous reactor configurations for the compounds studied. Disappearance rates were calculated, but mineralization rates were emphasized. Mineralization provides an unambiguous predictor of biodegradation, but disappearance kinetics are required for fate predictive equations.

17. Mineralization rates for the compounds studied were generally comparable over the batch, fill-and-draw, and continuous reactor configurations. However, disappearance rate kinetics were comparable only for batch and fill-and-draw systems and ranged from 1 to 3 orders of magnitude slower than those demonstrated in the steady-state continuous systems. The batch and fill-and-draw kinetic disappearance values compare well with data reported in the literature for specific compounds and BOD. Better experimental assays for disappearance rate quantification are needed. Once developed, the reasons for the wide variations in disappearance rates between reactor configurations can and should be studied.

18. Specific compound degraders represent less than 0.1% of the total viable biomass in all cases. This implies that a sub-population of a given biomass is responsible for initial bio-transformation but that metabolites are released to support the growth of the other viable organisms. This may suggest that quantification and control of that sub-population is critical for the treatment of a trace recalcitrant compound. In addition, measures of total biomass such as MLSS or MLVSS are not sufficiently descriptive for calculation of second-order rate constants and for comparisons across systems. Methods to improve the enumeration of the degradative population should be refined and used to further understand the importance of this biomass fraction in treatment of recalcitrant compounds in wastewater.

19. Laboratory-scale activated sludge systems were designed and operated to elucidate compound fates in a conclusive fashion. This included the use of radiolabeled substrate in conjunction with nonlabeled substrate and sampling of all streams of fate importance. Many of these procedures are substantially more difficult to implement in full-scale processes. Full-scale fate data cannot, therefore, be as precise or conclusive as the more controlled experiments. Methods to incorporate the transient feed flows and compositions common to full-scale plants into the lab-scale studies should be undertaken to quantify the scaling factors between the lab-scale and the full-scale data.

20. A coupled, algebraic, predictive fate equation is presented subject to assumptions and derivations herein. This equation should be verified for many compounds. Upon verification, this equation may be used to correct lab-, pilot-, and full-scale data for the effects of stripping and sorption and to develop corrected biological disappearance rate kinetics. Suitable existing data sources should be analyzed to generate these corrected rates, and this compilation should be made available.

21. Upon verification, the coupled equations may be used to estimate the fates of chemicals in a given plant or over the universe of plants, if ranges of system design variables can be estimated. If the probability distribution of the system design variables is known (and if the equations are verified) quantitative fate predictions can be made over groups of treatment processes for specific organic chemicals.

22. The coupled fate equations may be adapted to plug flow and batch processes, although the result will not incorporate a simple algebraic solution

and experimental methods for data collection (especially bio-oxidation) may require adaptation.

23. An example of organic compound fates is provided by assuming most probable, maximum, and minimum values for the equation variables and using biological rate constants calculated from other studies. Generally, the most probable values agree with the findings in this study, and the ranges agree with full-scale plant studies implemented by EPA. With all additional verification noted earlier, this deterministic predictive fate method has the potential for use in regulatory processes, fate quantification, and optimization of system design.

SECTION 3

EVALUATION OF THE EXISTING DATA BASE

SUMMARY

This section summarizes the literature review work performed during a preceding project, (Contract 68-03-3027, Work Assignment 1, Treatability of Organic Chemicals). A basic objective of this project was to determine if the treatability of organic compounds, such as the priority pollutants, could be predicted based on possible grouping of the chemicals by common physical properties, chemical structure, or other physical-chemical properties of the organic substrates. A major part of this project was to survey the literature to determine the present level of understanding of the relationships between chemicals and their treatment in activated sludge treatment processes. References collected and included in this effort are presented in Appendix A.

Treatability was analyzed in terms of the basic removal/conversion mechanisms of (1) bio-oxidation, (2) stripping, and (3) sorption. In some cases other mechanisms such as chemical oxidation, hydrolysis, or photolysis become important. The first three mechanisms were given priority in this study.

Treatability data on over 1000 specific organic chemicals were collected from the literature. The literature search primarily focused upon lab and pilot scale data because it was felt that such data, being more controlled than full-scale data, would be inherently of higher quality for predictive fate method development. Priority was also given to collecting data which might have been useful in quantifying treatability mechanisms in aerobic, suspended growth biological processes. Abstraction of data was divided into the areas of stripping, sorption, and bio-oxidation data.

Bio-oxidation data were classified according to the type of test method used to generate the data. The primary test method classification included the following:

- Biochemical oxygen demand test
- Flask tests
- Respirometric tests
- Activated sludge treatability studies

Since there were basic differences in the environments in which these tests were conducted, quantitative comparison of different studies in the literature was difficult and much of the data was of limited value in assessing the relative rates of biodegradation of various compounds. Test conditions and process operating parameters were also often not specified.

In order to evaluate the quality of the bio-oxidation data base, and to identify data generated on a consistent basis, a minimal set of screening criteria were established for each test method. These criteria included system parameters known to affect biodegradability test results as well as criteria to address data needs specific to this project, such as kinetic data and recognition and quantification of major treatability mechanisms.

Application of the screening criteria limited the database to approximately 25 compounds.

The alternative to this course of action would have been to attempt to correlate chemical structure and biodegradability for compounds that were tested by the same method and analytical protocol. This plan would essentially have limited comparisons to data reported within a single reference or a very limited number of references. There are a number of studies in which a large number of compounds were tested by the same protocol. A listing of these studies is presented in this chapter.

The researchers in this study and other investigators[18] found that the data base on stripping of organic chemicals from activated sludge treatment systems was limited to a very small number of compounds. The major studies which had been published at that time involving the quantification of stripping of organic chemicals from activated sludge systems were studies by Kincannon, Stover, and Ching[19] and Kincannon and Stover.[20] The scope and results of these studies are discussed in Section 10 of this report.

These studies indicated that several compounds which could be 80 to 100% stripped from nonbiological systems showed little or no stripping from activated sludge systems. These data indicated that the relative rates of biodegradation and stripping must be quantified if relative estimates of the treatability of individual compounds are to be accomplished.

The primary reference from which data on the sorption of organic compounds onto sludge was obtained was a field study on the fate of priority pollutants in publicly owned treatment works. This study was conducted by the EPA Effluent Guidelines Division. Although forty plants were eventually included in this survey, preliminary analytical results for twenty of these plants were available at the time this study was conducted.

Correlation coefficients were developed between influent concentration to the primary clarifier and loading on the primary sludge for each of fourteen compounds. (The term "loading" as used in this work is similar to the usage when referring to partitioning of chemical on solids such as activated carbon. In this context "loading" is the weight concentration of chemical per weight of solid. "Loading" in environmental engineering is a weight flux per unit volume of a biological process. The environmental engineering connotation will not be used in this report.) Correlation coefficients were also developed between secondary effluent concentration and the compound loading on the secondary sludge for three compounds. Only compounds for which five or more samples had measurable concentrations reported in both the aqueous phase and the sludge phase were used in the correlation analysis.

In general, correlations between aqueous phase concentrations and solid phase concentrations were poor. The poor degree of correlation may have been due to a variety of factors, such as differences in sludge characteristics from plant to plant and analytical variability when analyzing concentrations in the low ppb range.

BACKGROUND

Industrial wastewater biological treatment plants have been largely designed to achieve the reduction of organic pollution as measured by conventional analytical parameters such as biochemical or chemical oxygen demand, or total organic carbon (BOD, COD, or TOC) in process wastewaters. This philosophy was augmented by past EPA discharge regulations emphasizing plant performance standards based on removal of conventional analytical parameters. However, in recent years research emphasis has been placed on the identification and study of chemicals shown to accumulate in the environment or to possess otherwise hazardous properties. This increased interest in the fate of organic chemicals in the environment has created a great deal of interest in identifying the removal mechanisms of these compounds in biological wastewater treatment plants.

Convery, et al., pointed out that while the terms "removability" and "treatability" have commonly been used interchangeably, there is a justifiable reason to make a distinction between the two terms. Treatability is "a property or characteristic of the molecule...and is determined by specified procedures under controlled conditions and thus compounds can be compared or ranked relative to other compounds."[21] Treatability of a compound may be highly specific to process conditions. In our viewpoint, the term also implies an assessment of the mechanisms responsible for the removal of a compound from solution.

Removability is a more general term which implies only a change in concentration in the compound between the time it enters and leaves a process. Removability in biological treatment systems may involve several treatment mechanisms, including:

- Biological conversion
- Stripping
- Sorption to sludge
- Chemical conversion
- Photolysis
- Hydrolysis
- Precipitation

The first three of these mechanisms are generally acknowledged as the primary treatment mechanisms and were given priority in this study.

The major goal of this study was to be able to quantitatively predict how much of a chemical would be removed from a biological treatment system by each of these mechanisms. In order to make this prediction, data must be available on the kinetic rate for each removal mechanism. The kinetic rates will be a function not only of the physical and chemical properties of the specific compounds, but also of operating parameters of the experimental system such as temperature, pH, flow rate, chemical and microbial concentrations, etc. Therefore, in order to compare data from various studies, the data must be generated by similar protocols or the factors which are not constant must be included as variables in a predictive equation.

ANALYSIS OF BIO-OXIDATION DATA

The initial phase of Project No. 68-03-3027, Treatability of Organic Chemicals, involved the collection and evaluation of an extensive data base of biodegradability information for a wide range of organic chemicals. A concurrent effort involved the collection of data defining the degree of stripping and sorption onto biological solids in wastewater treatment processes.

A key criterion for selecting information for inclusion in the data base was the degree to which the three major conversion or transport mechanisms, i.e., biological oxidation, stripping, and sorption, could contribute to the removal of a compound from the test solutions. The second major criterion used in screening data for inclusion in the data base was the number of experimental factors involved in the test protocol. For example, data from batch-type laboratory experiments on single compounds were preferable to operating data from full-scale plants where variable flows, influent compositions, and system operating parameters complicate interpretation of the data.

Since an extremely large data base exists on the biodegradability of specific organic chemicals, several additional criteria were used as guidelines in screening data sources to be reviewed. Although much of the data that were collected did not meet all these criteria, priority was given to collecting and abstracting biological degradation data possessing as many of the following qualities as possible:

- Experimental procedure amenable to collection of kinetic data
- Experiment conducted in aqueous phase
- Experiment conducted in an aerobic environment
- Presence of a heterogeneous bacterial population
- Indication of the degree of primary biodegradation, e.g., conversion to carbon dioxide, water, and inorganic compounds
- Single test compound (sole carbon source)
- Degree of acclimation of microbial population specified
- Major environmental factors (pH, temperature, nutrients) specified

In order to satisfy the objectives of the study, biodegradability test results should permit a determination of both the rate of biodegradation as well as the extent. As with any chemical reaction, the rate of chemical conversion is dependent on the reactants and their concentrations. In biological treatment systems, the reactants are the specific waste components and the enzyme systems of the microorganisms. Therefore, the rate of any biodegradation reaction depends on the types of microorganisms present, their ability to generate enzymes catalyzing substrate conversion, the growth rate of the microbial population, the effect of metabolic byproducts, the concentration of the chemical substrate, and the effect of environmental factors such as temperature, pH, sufficient levels of dissolved oxygen, and presence of inorganic nutrients.

To develop a bio-oxidation predictive fate method (PFM), kinetic data either must be available over short increments of time or capable of being extrapolated from data collected over longer time periods. A basic conflict arises because activated sludge biological treatment systems generally operate with high concentrations of microorganisms and relatively short hydraulic residence times while most test methods for measuring biodegradation use low concentrations of microorganisms and long test durations.

Before trying to assess the quality of a data base derived from a large number of studies conducted for varying purposes, a brief discussion should be devoted to defining biodegradability, the techniques used for measuring biodegradability, and the major factors affecting each of these tests. Examination of these tests reveals basic differences in the environment under which these tests are conducted, making comparison of data collected by different test methods of limited value in assessing the relative biological stability of different compounds.

Three broad categories have been proposed as an operational definition of biodegradation:[22]

- Primary: molecular alteration to the extent that the compound no longer responds to the analytical technique being used
- Ultimate: conversion of the parent compound to water, carbon dioxide, biomass, and inorganic compounds (if elements other than carbon, hydrogen, and oxygen are present)
- Acceptable: conversion of the parent compound to the extent that undesirable properties are no longer manifest

In this work, the terms "disappearance" and "transformation" will be used synonymously with "primary biodegradation" and "mineralization" will be used interchangeably with "ultimate biodegradation." Since the last of the three definitions listed above is obviously subjective, data collection for this report focused on information that could be quantified according to one of the first two definitions. The primary methods that are used to measure biodegradability include:

- Specific analysis for the parent chemical compound
- Specific analysis for a product of biodegradation (such as chlorine)
- Non-specific analysis for a conventional parameter such as COD or TOC
- O_2 utilization by microorganisms metabolizing the compound
- CO_2 production by microorganisms metabolizing the compound
- Growth of microorganisms utilizing the compound as a sole source of carbon

In order to assess ultimate biodegradation, some type of analytical test must be employed which quantifies the total residual organic content or a material balance for the products of degradation.

Biodegradability test results reported in the literature and used in compiling this data base include:

- Biochemical oxygen demand test
- Warburg and other respirometric techniques
- Flask tests
- Activated sludge studies

The important features and limitations of these tests have been discussed in general terms by Barth and Bunch.[22] The following discussion reiterates many of their observations and discusses the limitations of each of these tests to accomplish the objective of this project, i.e., be able to distinguish and model the transport/conversion mechanisms of biodegradation, stripping, and sorption.

Biochemical Oxygen Demand Test

The biochemical oxygen demand test is an empirical test that may be used to determine the relative oxygen demand of organic compounds. The test consists of storing a test sample in the dark at 20°C for a specified time period and measuring the amount of oxygen utilized. Although standard procedures for performing BOD determinations are well documented,[23] variability of test protocol and analytical techniques still exists. For example, nitrification may or may not be inhibited and seed microorganisms may be obtained from domestic wastewater, natural waters receiving waste discharges, effluent from a biological treatment system, or acclimated seed developed in a laboratory. Because of these variations, caution has been expressed against the comparison of BOD values unless the results have been obtained under identical test conditions. However, the test does provide standardization of test procedures and reduction or elimination of compound disappearance by other fate mechanisms as outlined below:

- Standard bottle sizes specified
- Incubation temperature specified (20°C)
- Dilution water contains standard concentrations of inorganic nutrients
- Endogenous respiration recognized and corrected for by a standard procedure
- Photolysis of test compound and photosynthetic oxygen production avoided by incubating test solutions in the dark

- Stripping of volatile compounds minimized by conducting test in a closed container
- Sorption of test compound onto biological solids minimal due to low concentration of biological solids present
- Test results normally reported for a five-day or twenty-day test period

Despite the relative degree of standardization in performing BOD tests, widely varying results are reported for the same compound by various investigators. For example, BOD results presented in the literature have been tabulated by Heukelekian[24] and a portion of these results have been analyzed and are presented in Table 2. Compounds listed in Table 2 include all compounds reported by Heukelekian with five or more data points. As demonstrated by these data, wide ranges of values have been reported for each compound and the standard deviation for each compound is generally large compared to the average. Several factors are most likely responsible for a large degree of this variance. Differences in degree of acclimation of the microbial population may cause significant differences in oxygen consumption in the BOD test. Data presented by Price[25] are abstracted in Table 3 and serve to illustrate this point. For 13 of the 16 compounds listed in Table 3, the 5-day BOD is greater for the tests conducted with acclimated seed than for the test conducted with nonacclimated seed. Even after a 20-day incubation period, 13 of the 16 systems still show a greater O_2 uptake for the acclimated systems. For the 16 compounds, the average of the theoretical carbonaceous oxygen demand is 66% and 70% greater for the acclimated than for the nonacclimated systems at 5 and 20 days, respectively. These data indicate that significant time periods can be required for acclimation and that acclimation can significantly increase the degree of biodegradation measured by the BOD test protocol.

TABLE 2. VARIABILITY OF BIOCHEMICAL OXYGEN DEMAND OF ORGANIC COMPOUNDS[a]

Compound	Number of data points	BOD_5 (g/g compound)			
		Minimum	Average	Maximum	Standard deviation
Acetic acid	9	0.34	0.62	0.88	0.18
Sodium acetate	7	0.11	0.33	0.67	0.18
Methyl alcohol	11	0.76	0.86	1.12	0.11
Ethyl alcohol	12	0.93	1.25	1.67	0.23
Glycerol	6	0.64	0.75	0.98	0.14
Formaldehyde	5	0.33	0.57	1.06	0.30
Acetone	9	0.05	0.89	1.63	0.55
Glucose	10	0.50	0.80	1.01	0.45
Ethyl acetate	6	0.50	0.66	1.13	0.29
Phenol	5	1.40	1.76	2.10	0.25

[a] See Reference 24.

TABLE 3. COMPARISON OF BIOCHEMICAL OXYGEN DEMAND OF ORGANIC CHEMICALS AS INFLUENCED BY ACCLIMATION OF SEED MICROORGANISMS[a]

Compound	Percent of theoretical carbonaceous oxygen demand							
	Nonacclimated				Acclimated			
	5 Days	10 Days	15 Days	20 Days	5 Days	10 Days	15 Days	20 Days
Benzene	24	27	24	29	58	67	76	80
Di-decylphthalate	1	1	1	0	7	10	3	7
Diethylene glycol	5	8	13	30	43	55	61	67
Diethylenetriamine	4	4	4	0	23	46	51	70
Ethyl acrylate	28	32	32	35	66	74	76	79
Ethylenediamine	24	44	55	47	36	45	56	70
Ethyl hexyl acrylate	0	24	29	30	9	15	23	40
Formic acid	48	54	66	68	51	47	39	60
Ethylene glycol	2	29	47	48	55	85	88	90
Isobutanol	64	73	76	72	46	71	75	81
Isodecanol	24	44	42	39	14	26	45	32
Isopropyl acetate	61	72	74	76	47	53	54	62
Tetraethylene glycol	4	10	15	22	9	58	71	88
Toluene	53	62	70	80	73	74	80	86
Triethylene glycol	4	10	17	24	32	64	77	86
Vinyl acetate	34	34	31	32	62	70	66	72

[a] See Reference 25.

Warburg and Other Respirometric Tests

A variety of instruments have been used to measure either O_2 uptake or CO_2 production. The most widely used of these instruments is the Warburg respirometer. Respirometric tests commonly measure O_2 uptake manometrically by the pressure of the gas in a closed system. Carbon dioxide produced during the course of biodegradation is typically absorbed by a strong base such as sodium hydroxide or potassium hydroxide.

Warburg tests have several advantages over conventional BOD tests. Substrate concentrations that can be tested via the BOD test are limited by the solubility of oxygen in the test solution. However, Warburg tests can be performed for a much broader range of substrate concentrations since oxygen is transferred into solution during the test. Likewise, measurable concentrations of sludge may be used so that the effects of sludge concentration as well as substrate concentration may be investigated.

Gellman[26] investigated the effects of both substrate concentration and sludge concentration on the oxidation of formaldehyde in a Warburg apparatus. These data are presented in Table 4 and Table 5, respectively. The stoichiometric equation for the oxidation of formaldehyde is as follows:

$$HCHO + O_2 \rightarrow CO_2 + H_2O$$

On a weight basis, this equation indicates that 1.07 mg of oxygen will be required for the oxidation of 1.00 mg of formaldehyde. Since the data reported in Tables 4 and 5 were generated in a wastewater medium and values of O_2 uptake due to endogeneous respiration were not reported, no estimate of the theoretical oxygen uptake could be made. However, the data in Table 4 serve to illustrate the point that O_2 uptake may be a function of substrate concentration only within limited ranges. These data indicate an abrupt breakpoint in toxicity level for formaldehyde somewhere between concentrations of 130 mg/L and 175 mg/L. However, following acclimation of the microbial population to the formaldehyde substrate, the authors reported 95% oxidation of formaldehyde at concentrations up to 1750 mg/L within 24 hours.

Data in Table 5 indicate that oxygen uptake is a function of sludge concentration but that the function is not linear. Although this function may be linear over certain ranges of sludge concentration, the rate of O_2 uptake may be influenced by several limiting factors. For example, the ratio of O_2 uptake to sludge concentration is approximately linear for the two cases with volatile suspended solids (VSS) concentrations of 2090 mg and 1045 mg/L for the first eight hours of the test. However, at the data points taken at time equal to 20 hours, it appears that substrate limitations may control the amount of O_2 consumption. A similar comparison of the data from the experiments with VSS concentrations of 2090 mg/L and 4180 mg/L does not indicate a linear relationship between sludge concentrations and oxygen uptake. One possible explanation for this non-linearity may be that oxygen mass transfer across the gas/liquid interface may be the factor that controls oxygen uptake. Similar mass transfer limitations have been observed by Sato[27] and Kirsh.[28]

TABLE 4. EFFECT OF CONCENTRATION ON OXIDATION OF FORMALDEHYDE AS MEASURED BY CUMULATIVE OXYGEN UPTAKE (mg/L)[a]

Time (days)	Formaldehyde concentration (mg/L)			
	45	90	130	175
1	0	0	0	0
2	15	0	0	0
3	21	72	43	0
3.25	23	81	92	0
4	25	89	112	0
7	28	90	135	0

[a] See Reference 26.

TABLE 5. EFFECT OF SLUDGE CONCENTRATION ON OXIDATION OF FORMALDEHYDE AS MEASURED BY OXYGEN UPTAKE (mg/L)[a]

Time (hr)	Sludge Concentration (mg/L VSS)			
	4180	2090	1045	418
0.5	121	89	47	32
1.0	192	153	79	50
2.5	325	250	133	83
4.0	531	493	224	144
8.0	838	850	534	322
20.0	913	968	815	766

[a] See Reference 26.

Several investigators have attempted to develop biodegradability indicators based on a percentage of theoretical carbonaceous oxygen demand as measured by Warburg respirometry. The examples which have been discussed indicate that development of such indices must include an evaluation of substrate concentration-induced toxicity effects, oxidizable substrate limitations, and oxygen mass transfer limitations. Test data over wide ranges of substrate and sludge concentrations will be required to adequately assess these limitations.

One of the chief limitations of any respirometric biodegradation test is the interpretation of oxygen uptake or carbon dioxide production due to the endogenous respiration of the seed microorganisms. Endogenous respiration is usually defined as oxygen uptake by cells resulting from the oxidation of stored food materials.[29] During extended periods of starvation, oxidation of vital cell components might also occur. A key question is to what degree endogenous respiration continues in the presence of an oxidizable exogenous substrate. Malaney contends that the answer to this question is both substrate-specific and microbial population-specific.[29]

When oxygen uptake in the presence of an exogenous substrate exceeds the oxygen uptake by an endogenous control, it has generally been accepted that the exogenous substrate is being degraded. However, cases have been reported of a substrate promoting oxygen uptake without the substrate itself being degraded.[30] In either case, inability to ascertain the degree of endogenous respiration in the presence of an exogenous substrate precludes a rigorous calculation of the percent of substrate removal.

If oxygen uptake in the presence of an exogenous substrate is less than an endogenous control, the compound is normally considered to be inhibitory. However, this interpretation of results assumes that endogenous respiration continues unhindered in the presence of an exogenous substrate when in reality it may not occur or may occur at some reduced rate.

In order to assess the degree of removal of specific compounds, Warburg measurements should ideally be supplemented with specific analytical determination for the substrate. Such combinations of tests can be revealing, as demonstrated by data that have been reported by Baird[31] and are abstracted in Table 6. Warburg tests were run for eight aromatic amines and for a control flask which contained sludge with no additional carbon sources added. All of the aromatic amines with the exception of 2,4-dimethylaniline depressed oxygen uptake to levels below those measured in the control flask. These data indicate that the compounds are toxic or inhibitory to biological respiration. However, GLC analysis at the end of each Warburg test indicated reductions in the concentrations of the parent compounds ranging from 27 to 100%, indicating that inhibition was due to toxic metabolites rather than the parent compound.

TABLE 6. COMPARISON OF BIODEGRADABILTY OF AROMATIC AMINES AS MEASURED BY WARBURG TESTS AND GAS-LIQUID CHROMATOGRAPHY ANALYSIS FOR PARENT COMPOUND[a]

Compound[b]	Percent of endogenous respiration as measured by Warburg test[c]	Percent conversion of compound as measured by GLC[c]
Aniline	83	54±5
3,5-Dimethylaniline	68	53±5
2,6-Dimethylaniline	82	27±2
2,4-Dimethylaniline	102	36±1
Benzidine	88	90±3
o-Toluidine	94	100±0
Bianisidine	96	100±1
Tetramethylbenzidine	64	72±0

[a] See Reference 31.

[b] All compounds except tetramethylbenzidine initially present at 20 mg/L. Tetramethylbenzidine dosed at 50 mg/L.

[c] Test results at end of 6 hours.

Respirometric techniques utilizing compounds labeled with radioactive carbon-14 have been developed which can eliminate some of the uncertainty associated with the interpretation of respirometric data. One technique involves capturing and measuring the radioactive carbon dioxide released from the biological oxidation of the test compound. Radioactive and nonradioactive CO_2 can be distinguished, allowing an accurate calculation of the percent conversion of the original compound to CO_2.

Flask Tests

A wide variety of flask test methodologies are reported in the literature. Flask tests are generally conducted by spiking a sample with a test compound and measuring the disappearance of the test compound as a function of time. Flasks may be open or closed, static, shaken, mechanically agitated, or aerated. Tests conducted in open flasks have the potential for compound losses through stripping as well as biodegradation. Data presented by Kirsch indicate that the degree of agitation of the test solution can affect the mass transfer rate of a compound between an aqueous phase and the cell and lead to a significant difference in oxidation rate.[28]

A number of studies have investigated the effects of various aqueous media and seed inocula on the biodegradability of organic compounds. Brown studied the biodegradation of acrylamide monomer spiked into a variety of natural and wastewater samples.[32] Data from this study illustrate the influence of the aqueous medium on the rate of the biodegradation process and are shown in Table 7. These data show significant differences in the lag time before the beginning of biodegradation and the time required for 50% conversion of the acrylamide monomer. The differences may be considered due to both the numbers and types of microorganisms present in the sample and the presence of other organic and inorganic compounds.

TABLE 7. EFFECT OF AQUEOUS MEDIUM ON THE BIODEGRADATION OF ACRYLAMIDE MONOMER[a]

Medium	Lag time before beginning of measurable biodegradation (hr)	Time required for 50% biodegradation (hr)
Seawater	160	190
Seawater	160	190
Estuarine water	105	130
River water	40	65
River water	30	55
River water	400	550
Peat bog water	50	70
Industrial wastewater	70	110
Industrial wastewater	160	250
Industrial wastewater	0	4
Sewage effluent	50	70

[a] See Reference 32.

Tabak and co-workers used a relatively simple static flask biodegradability screening test to group the priority pollutants based on their susceptibility to bio-oxidation.[33] The static flask tests were conducted at concentrations of 5 mg/L and 10 mg/L for each compound. Tests were conducted in a medium consisting of BOD dilution water with 5 mg/L yeast extract, and a settled domestic wastewater inoculum. Each culture was incubated for 7 days at 25°C in the dark. The original culture was followed by three subcultures of 7 days duration each in order to follow the progressive acclimation of the microorganisms to the substrate. Controls with no biomass were used to assess volatilization losses for all halogenated aliphatics.

A summary of this work is presented in Table 8. Compounds are ranked in order of decreasing resistance to bio-oxidation. For compounds with no reported volatilization data, the average percent biodegradation is the average of the eight observations. For compounds with volatilization data reported, the average percent biodegradation has been adjusted for volatilization losses by the following two equations:

$$P_{Bi} = (P_T - P_V)/(100 - P_V) \times 100 \qquad \text{Eqn. 15}$$

$$A_B = \Sigma P_{Bi}/N \qquad \text{Eqn. 16}$$

where:

P_{Bi} = percent bio-oxidized for a given culture,
P_T = percent total loss for a given culture,
P_V = percent volatilized,
A_B = average percent bio-oxidized, and
N = number of cultures.

As shown by these data, the degree of biodegradation is significantly affected by the acclimation of the microbial population to the test compound. Furthermore, the degree of biodegradation as measured in this test may be greater or may decline after an acclimation period. Increased degrees of biodegradation following an acclimation period may be attributable to shifts in the distribution of species in a microbial population and the induction of enzymes within a population. Decreasing degrees of biodegradation after acclimation procedures may possibly be due to a buildup of toxic metabolites within the microbial population, the loss of a metabolically efficient population, or the appearance of protozoa or bacteriophages that kill many of the viable cells.

Laboratory-Scale and Pilot-Scale Activated Sludge Methods

Laboratory-scale activated sludge studies may be operated in either batch or continuous modes. The continuous system can be used to simulate a complete mix, full-scale process while batch (or fill-and-draw) techniques more closely approximate plug flow conditions. Laboratory-scale activated sludge studies more nearly represent plant operating conditions with respect to biomass levels and oxygen transfer conditions than do the other common biodegradability test methods. However, the method has one serious drawback in performing fate studies since what is normally measured is compound removability rather than biodegradability. Removal of organic compounds in activated sludge units has historically been attributed to biodegradation, when in fact stripping and sorption onto sludge may also be significant removal mechanisms.

There have been a number of recent studies in which two or more of the removal mechanisms were investigated.[4,6,19,20,34,35] Results of these studies are discussed in Section 10.

TABLE 8. STATIC FLASK TEST RESULTS

COMPOUND	AVERAGE TOTAL LOSS OF TEST COMPOUND IN 7 DAYS								% VOLATILIZED		AVERAGE % BIODEG
	5 mg/1				10 mg/1				@ 5 mg/1	@ 10 mg/1	
	ORIG	FIRST	SEC	THIRD	ORIG	FIRST	SEC	THIRD			
A-BHC-ALPHA HEXACHLOROCYCLOHEXANE	0	0	0	0	0	0	0	0			0.00
ALDRIN	0	0	0	0	0	0	0	0			0.00
B-BHC ALPHA HEXACHLOROCYCLOHEXANE	0	0	0	0	0	0	0	0			0.00
BIS-(2-CHLOROETHOXY) METHANE	0	0	0	0	0	0	0	0			0.00
CHLORODANE	0	0	0	0	0	0	0	0			0.00
DDD P,P'	0	0	0	0	0	0	0	0			0.00
DDE P,P'	0	0	0	0	0	0	0	0			0.00
DDT P,P'	0	0	0	0	0	0	0	0			0.00
DIELDRIN	0	0	0	0	0	0	0	0			0.00
ENDOSULFAN SULFATE	0	0	0	0	0	0	0	0			0.00
ENDOSULFAN-ALPHA	0	0	0	0	0	0	0	0			0.00
ENDOSULFAN-BETA	0	0	0	0	0	0	0	0			0.00
ENDRIN	0	0	0	0	0	0	0	0			0.00
HEPTACHLOR	0	0	0	0	0	0	0	0			0.00
HEPTACHLOR EPOXIDE	0	0	0	0	0	0	0	0			0.00
LINDANE	0	0	0	0	0	0	0	0			0.00
D-BHC-DELTA HEXACHLOROCYCLOHEXANE	0	0	0	0	0	0	0	0			0.00
PCB-1248	0	0	0	0	0	0	0	0			0.00
PCB-1260	0	21	0	0	0	6	0	0			3.38
4-BROMODIPHENYL ETHER	0	19	36	0	0	0	10	0			8.12
TRICHLOROFLUOROMETHANE	59	61	65	73	38	40	43	45	58.00	37.00	11.31
1,1,2,2-TETRACHLOROETHANE	0	12	27	29	0	11	20	23	7.00	0.00	12.13
4-CHLORODIPHENYL ETHER	0	32	36	1	0	28	5	0			12.75
PCB-1254	11	42	15	0	10	26	0	0			13.00
HEXACHLOROBENZENE	56	30	8	5	21	3	0	0			15.37
CHLORODIBROMOMETHANE	25	28	35	39	10	12	21	25	16.00	3.00	16.59
1,2-BENZANTHRACENE	16	41	35	0	0	38	23	0			19.12
1,1,2-TRICHLOROETHANE	6	30	38	44	0	27	30	39	15.00	3.00	19.35
BROMOFORM	11	22	40	48	4	14	31	35	0.00	0.00	25.62
4,6-DINITRO-O-CRESOL	52	58	56	51	0	5	11	14			30.88

TABLE 8. (CONTINUED

COMPOUND	AVERAGE TOTAL LOSS OF TEST COMPOUND IN 7 DAYS								% VOLATILIZED		AVERAGE % BIODEG
	5 mg/1				10 mg/1						
	ORIG	FIRST	SEC	THIRD	ORIG	FIRST	SEC	THIRD	@ 5 mg/1	@ 10 mg/1	
BENZENE	49	100	100	100	37	95	100	95			84.50
N-NITROSODIPHENYLAMINE	87	100	92	100	47	63	95	98			85.25
CHLOROBENZENE	89	100	100	100	30	77	100	100			87.00
1,1-DICHLOROETHYLENE	78	100	100	100	45	100	100	100	24.00	15.00	88.29
2,4-DINITROPHENOL	60	100	100	100	68	100	100	100			91.00
ETHYLBENZENE	100	100	100	100	69	78	87	100			91.75
BIS-(2-CHLOROISOPROPYL) ETHER	85	100	100	100	63	100	100	100			93.50
P-CHLORO-M-CRESOL	78	100	100	100	76	100	100	100			94.25
ACENAPHTHYLENE	100	97	96	98	94	91	93	93			95.25
CARBON TETRACHLORIDE	87	100	100	100	80	100	100	100	23.00	5.00	95.26
2-CHLOROPHENOL	86	100	100	100	83	100	100	100			96.13
NITROBENZENE	100	100	100	100	87	97	100	100			98.00
PHENOL	96	100	100	100	97	100	100	100			99.13
2,4-DICHLOROPHENOL	100	99	98	100	99	99	100	99			99.25
ACENAPHTHENE	95	100	100	100	100	100	100	100			99.38
2,4-DIMETHYLPHENOL	100	100	100	100	99	100	100	100			99.88
PCB-1221	100	100	100	100	99	100	100	100			99.88
2,4,6-TRICHLOROPHENOL	100	100	100	100	100	100	100	100			100.00
2-CHLORO NAPHTHALENE	100	100	100	100	100	100	100	100			100.00
2-NITROPHENOL	100	100	100	100	100	100	100	100			100.00
4-NITROPHENOL	100	100	100	100	100	100	100	100			100.00
ACROLEIN	100	100	100	100	100	100	100	100			100.00
ACRYLONITRILE	100	100	100	100	100	100	100	100			100.00
BIS-(2-CHLOROETHYL) ETHER	100	100	100	100	100	100	100	100			100.00
BROMOCHLOROMETHANE	100	100	100	100	100	100	100	100	11.00	3.00	100.00
BUTYL BENZYL PHTHALATE	100	100	100	100	100	100	100	100			100.00
DI-N-BUTYL PHTHALATE	100	100	100	100	100	100	100	100			100.00
DIETHYL PHTHALATE	100	100	100	100	100	100	100	100			100.00
DIMETHYL PHTHALATE	100	100	100	100	100	100	100	100			100.00
HEXACHLORO-1,3-BUTADIENE	100	100	100	100	100	100	100	100	1.00	0.00	100.00

TABLE 8. (CONTINUED)

COMPOUND	AVERAGE TOTAL LOSS OF TEST COMPOUND IN 7 DAYS								% VOLATILIZED		AVERAGE
	5 mg/l				10 mg/l						
	ORIG	FIRST	SEC	THIRD	ORIG	FIRST	SEC	THIRD	@ 5 mg/l	@ 10 mg/l	% BIODEG
FLUORANTHENE	0	47	100	100	0	0	0	0			30.88
N-NITROSO-DI-N-PROPYLAMINE	27	37	47	50	0	8	40	40			31.12
1,2-DICHLOROETHANE	26	41	54	63	20	35	51	53	27.00	5.00	31.30
PCB-1242	37	41	47	66	34	33	15	0			34.12
CHRYSENE	6	65	53	59	0	30	34	38			35.62
1,4-DICHLOROBENZENE	55	61	34	16	37	54	29	0			35.75
PCB-1016	44	47	46	48	22	46	20	13			35.75
1,2-DICHLOROBENZENE	45	66	48	29	20	59	32	18			39.62
1,2,4-TRICHLOROBENZENE	54	70	59	24	43	54	14	0			39.75
DICHLOROBROMOMETHANE	35	54	56	59	34	44	46	51	8.00	1.00	44.96
2,6-DINITROTOLUENE	82	55	47	29	57	49	35	13			45.87
2,4-DINITROTOLUENE	77	61	50	27	50	49	44	23			47.62
PYRENE	71	100	100	100	11	2	0	0			48.00
1,3-DICHLOROBENZENE	59	69	39	35	58	67	31	33			48.87
TETRACHLOROETHYLENE	45	54	69	87	30	41	67	84	23.00	16.00	49.97
PENTACHLOROPHENOL	19	68	100	100	16	0	0	99			50.25
1,1,1-TRICHLOROETHANE	29	64	76	83	23	53	68	75	27.00	7.00	50.33
ANTHRACENE	43	70	84	92	26	30	40	51			54.50
BIS-(2-ETHYL HEXYL) PHTHALATE	0	43	80	95	0	47	89	93			55.87
FLUORENE	82	53	67	77	65	43	38	45			58.75
1,2-DICHLOROETHYLENE-CIS	54	76	79	86	43	75	78	84	34.00	27.00	59.57
TRICHLOROETHYLENE	64	73	82	87	38	56	76	84	29.00	22.00	60.05
DI-N-OCTYL PHTHALATE	0	79	89	94	0	42	93	92			61.13
1,2-DIPHENYLHYDRAZINE	80	73	66	77	72	35	40	40			62.87
1,3-DICHLOROPROPYLENE	55	65	75	85	54	64	69	84	19.00	7.00	64.14
1,2-DICHLOPROPANE	42	70	79	89	36	61	69	81	3.00	0.00	65.41
1,1-DICHLOROETHANE	50	78	85	91	29	74	79	83	19.00	4.00	67.61
1,2-DICHLOROETHYLENE-TRANS	67	79	88	95	40	76	81	93	33.00	26.00	68.17
CHLOROFORM	49	85	92	100	46	70	80	100	24.00	6.00	74.00
2-CHLOROETHYL VINYL ETHER	76	75	100	100	52	68	100	100			83.88

TABLE 8. (CONTINUED)

COMPOUND	AVERAGE TOTAL LOSS OF TEST COMPOUND IN 7 DAYS								% VOLATILIZED		AVERAGE % BIODEG
	5 mg/l				10 mg/l				@ 5 mg/l	@ 10 mg/l	
	ORIG	FIRST	SEC	THIRD	ORIG	FIRST	SEC	THIRD			
HEXACHLOROCYCLOPENTADIENE	100	100	100	100	100	100	100	100	0.00	0.00	100.00
HEXACHLOROETHANE	100	100	100	100	100	100	100	100	0.00	0.00	100.00
ISOPHORONE	100	100	100	100	100	100	100	100			100.00
METHYLENE CHLORIDE	100	100	100	100	100	100	100	100	25.00	6.00	100.00
NAPHTHALENE	100	100	100	100	100	100	100	100			100.00
PCB-1232	100	100	100	100	100	100	100	100			100.00
PHENANTHRENE	100	100	100	100	100	100	100	100			100.00
TOLUENE	100	100	100	100	100	100	100	100			100.00

RESULTS AND DISCUSSION

Bio-oxidation Data Base Quality

The preceding subsection discussed the strengths and limitations of the various tests used for quantifying biodegradability. It may be concluded from the information presented that data generated by different test procedures cannot be compared on a quantitative basis. For any given test procedure, several system parameters influence biodegradability results. Any model of biodegradability must either utilize data that are generated on a consistent basis or must account for the system parameters as variables in the model.

Collection of existing biodegradation data in this study was originally conceptualized as a two-stage effort. The purpose of the first stage was to assess the quality, quantity, and types of available biodegradation data. Information collected in the first phase is shown in Table 9. An example of a portion of the data base is shown in Table 10.

At the conclusion of the initial data collection phase, the data base was evaluated to select specific references for the second stage of data collection. Data collected during the second stage would define the degree and rate of biodegradability of specific compounds. For example, a data record from the first phase of data collection might have indicated a Warburg respirometer test on acetic acid in which kinetic data were reported in units of mg/L of oxygen uptake as a function of time. The second phase of data collection would have recorded the actual milligrams per liter of oxygen consumed at discrete time increments as well as the oxygen consumed by endogenous respiration of the microbiological culture.

Data collected during the first stage included biodegradabilty information for 1037 different compounds with 2950 separate data records. A breakdown of the number of data records for each test method is as follows:

Unknown	61
Biochemical oxygen demand	607
Flask tests	799
Laboratory-scale activated sludge	326
Laboratory-scale trickling filter	15
Warburg respirometer tests	1039
Other respirometric tests	103

In order to assess the quality of the data base, a set of criteria was applied to all records for the different types of tests. These criteria included some of the important system parameters known to affect biodegradability test results. The criteria also addressed some data needs specific to this project, such as the availability of kinetic data. The criteria applied to each test method are listed in Table 11.

Results of the data base quality assessment are presented in Table 12. Since a very limited number of the data records located passed all the data

TABLE 9. COMPONENTS OF BIO-OXIDATION DATA BASE

Data item	Description of data item
Reference	Classified as original (primary) and assigned a document number
Compound name, test method, test compound concentration, analytical parameter measured, analytical technique	BOD, respirometric, flask test, activated sludge study, compound concentration, BOD, COD, TOC, CO_2 production, O_2 uptake, NO_3 production, Cl production, GC, HPLC, GCMS, LC, manometery, spectrophotometry, colorimetry, scintillation counting, ion specific probe
Concentration of microorganisms at beginning of test	MLSS, MLVSS
Microbiological culture type	Designated as single culture or mixed culture
Degree of acclimation of microorganisms to test compound	Number of days of acclimation
Aqueous medium used in test	Wastewater, natural water from river or lake, seawater, synthetic (tap water + minerals and organic nutrients)
Source of microorganisms used as inoculum	Specific culture, wastewater, seawater, soil
Temperature	Degrees °C
pH, Availability of kinetic data	Sufficient experimental data reported to derive kinetic parameters for biodegradation of compound under the conditions of the test

(Continued)

TABLE 9. (CONTINUED)

Data item	Description of data item
Biodegradation test units	Code specifying the format of the biodegradabilty test results; for example, Code A1 represents O_2 uptake (mg/L) with a correction for endogeneous respiration; Code A2 represents O_2 uptake (mg/L) without a correction for endogeneous respiration; a total of 27 unique sets of test units were identified in abstracting the bio-oxidation data
Test duration	Time duration of biodegradability test; for batch-type test, duration was considered to be the time from the introduction of the test compound to the microorganisms to the time that the last data was collected; for continuous flow tests, test duration was the period of time that continuous flow reactors were operated
Stripping and/or sorption	Presence of stripping or sludge sorption data or experimental and analytical controls to minimize or correct for loss of test compound due to stripping and/or sorption

TABLE 10. EXAMPLE OF BIODEGRADABILITY DATA BASE

							TEST COMPOUND		MICROBIOLOGICAL DATA					ENVIRONMENTAL COND				TEST RESUL				
REC #	DOC #	PRI OR SEC REF	COMPOUND	TEST METH	PARAM MEAS	ANAL TECH	CONC (MG/L)	MEAS AS	CONC (MG/L)	MEAS AS	CULT	AC	AC TIME (DAYS)	MEDIUM	INOC	TEMP (C)	pH	KIN DATA	TEST UNIT	TEST DUR	TEST DUR UNIT	ADS AND STR
1987	4039	P	PENTACHLOROPHENOL	FL	COMP	LC								SYN				Y	C1	90	DAYS	
1988	4039	P	PENTACHLOROPHENOL	FL	COMP	LC								SV				Y	C1	45	DAYS	
1989	4039	P	PENTACHLOROPHENOL	FL	COMP	LC								SV				Y	C1			
2722	5028	P	PENTACHLOROPHENOL	LSAS	COMP		20	COMP			MIXED	Y	90	WW	WW			Y	C2			AS
2723	5028	P	PENTACHLOROPHENOL	LSAS	COMP		20	COMP			MIXED	Y	90	WW	WW			Y	C2			AS
2724	5028	P	PENTACHLOROPHENOL	LSAS	COMP		20	COMP			MIXED	Y	90	WW	WW			Y	C2			AS
2725	5028	P	PENTACHLOROPHENOL	LSAS	COMP		20	COMP			MIXED	Y	90	WW	WW			Y	C2			AS
2726	5028	P	PENTACHLOROPHENOL	WAR	CO2	SCINT	20	COMP			MIXED	Y	90	WW	WW			Y	B3	12	HRS	AS
1204	4016	P	PENTACHLOROPHENOL	WAR	CO2	SCINT	30	COMP	2080	MLVSS	MIXED	Y	120	SYN	SOIL	25		Y	B3	46	HRS	
1206	4016	P	PENTACHLOROPHENOL	WAR	CO2	SCINT	30	COMP	2080	MLVSS	MIXED	Y	120	SYN	SOIL	25		Y	B3	46	HRS	
1208	4016	P	PENTACHLOROPHENOL	WAR	CO2	SCINT	30	COMP	2080	MLVSS	MIXED	Y	120	SYN	SOIL	25		Y	B3	46	HRS	
1210	4016	P	PENTACHLOROPHENOL	WAR	CO2	SCINT	30	COMP	2080	MLVSS	MIXED	Y	120	SYN	SOIL	25		Y	B3	46	HRS	
1213	5008	P	PENTACHLOROPHENOL(SODIUM SALT)	LSAS	COMP	SPECT	100	COMP			MIXED	Y		SYN	SOIL	26	7.0	N	C1	4	DAYS	
4098	8011	P	2,3,4,5,6-PENTAFLUORODIPHENYLMETHANE	LSAS	COMP	GC	1	COMP			MIXED	Y			WW			N	C1	7	DAYS	AS
4099	8011	P	2,3,4,5,6-PENTAFLUORODIPHENYLMETHANE	LSAS	COMP	GC	3.	COMP			MIXED	Y			WW			N	C1	7	DAYS	AS
4100	8011	P	2,3,4,5,6-PENTAFLUORODIPHENYLMETHANE	LSAS	COMP	GC	10	COMP			MIXED	Y			WW			N	C1	7	DAYS	AS
1830	4004	P	PENTANAL	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1831	4004	P	PENTANAL	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1832	4004	P	PENTANAL	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1214	4020	P	PENTANE	WAR	O2	MANOM	500	COMP	2500	MLVSS	SNGLE	N		SYN	CULT	20		Y	A1	7	DAYS	S
1650	4005	P	PENTANE	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1651	4005	P	PENTANE	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1652	4005	P	PENTANE	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1866	4004	P	2,3-PENTANEDIONE	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1867	4004	P	2,3-PENTANEDIONE	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1868	4004	P	2,3-PENTANEDIONE	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1869	4004	P	2,4-PENTANEDIONE	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1870	4004	P	2,4-PENTANEDIONE	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1871	4004	P	2,4-PENTANEDIONE	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1215	4020	P	1-PENTANETHIOL	WAR	O2	MANOM	500	COMP	2500	MLVSS	SNGLE	N		SYN	CULT	20		Y	A1	7	DAYS	S
3074	8006	P	PENTANOIC ACID	BOD	O2	PROBE	2	COMP			MIXED	N		SYN	WW	25	7.0	Y	A1	30	DAYS	
3075	8006	P	1-PENTANOL	BOD	O2	PROBE	2	COMP			MIXED	N		SYN	WW	25	7.0	Y	A1	30	DAYS	
1746	4004	P	1-PENTANOL	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1747	4004	P	1-PENTANOL	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
1748	4004	P	1-PENTANOL	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	
3096	8006	P	2-METHYL-1-PENTANOL	BOD	O2	PROBE	2	COMP			MIXED	N		SYN	WW	25	7.0	Y	A1	30	DAYS	
3097	8006	P	3-METHYL-1-PENTANOL	BOD	O2	PROBE	2	COMP			MIXED	N		SYN	WW	25	7.0	Y	A1	10	DAYS	
3098	8006	P	4-METHYL-1-PENTANOL	BOD	O2	PROBE	2	COMP			MIXED	N		SYN	WW	25	7.0	Y	A1	30	DAYS	
3099	8006	P	1,1-DIMETHYL-1-PENTANOL	BOD	O2	PROBE	2	COMP			MIXED	N		SYN	WW	25	7.0	Y	A1	30	DAYS	
4000	8006	P	2,2-DIMETHYL-1-PENTANOL	BOD	O2	PROBE	2	COMP			MIXED	N		SYN	WW	25	7.0	Y	A1	30	DAYS	
4001	8006	P	2,3-DIMETHYL-1-PENTANOL	BOD	O2	PROBE	2	COMP			MIXED	N		SYN	WW	25	7.0	Y	A1	30	DAYS	
1216	4020	P	2-PENTANONE	WAR	O2	MANOM	500	COMP	2500	MLVSS	SNGLE	N		SYN	CULT	20		Y	A1	7	DAYS	S
1857	4004	P	2-PENTANONE	WAR	O2	MANOM	500	COMP	2500	MLSS	MIXED	N		WW	WW	20		Y	A1	24	HRS	

quality criteria, a more detailed analysis was conducted for each criterion. The results of these analyses are presented in Tables 13 through 17.

TABLE 11. DATA QUALITY EVALUATION CRITERIA

	Test method			
Criteria	Biochemical oxygen demand	Flask tests	Warburg and other respirometric tests	Laboratory-scale activated sludge
Record from primary reference	X	X	X	X
Compound concentration specified	X	X	X	X
Sludge concentration specified			X	X
Mixed microbial population	X	X	X	X
Acclimated microbial population	X	X	X	X
Temperature specified	X	X	X	X
Kinetic data	X	X	X	X
Corrected for endogenous respiration	X		X	
Corrected for stripping and sorption				X

TABLE 12. RESULTS OF DATA QUALITY ASSESSMENT

Test method	Number of data records passing criteria	Number of compounds
Biochemical oxygen demand	1	1
Flask tests	3	2
Laboratory-scale activated sludge	3	2
Warburg and other respirometric tests	50	22

The initial objective of this research project was to relate the fate mechanisms of organic chemicals in biological treatment processes to the structure of the molecules to develop a STPFM. The data base collected on biodegradability of organic compounds demonstrated that the results of biodegradability tests were often as much a function of the random conditions

TABLE 13. DISTRIBUTION OF BIODEGRADABILITY INDICATOR UNITS AS A FUNCTION OF TEST METHOD

Test method	Number of records for each biodegradation test unit[a]																										
	A1	A2	A3	A4	A5	A6	A7	A9	A10	A11	A12	B1	B2	B3	B4	B5	C1	C2	D1	D2	E1	E2	F1	G1	H1	I1	J1
Biochemical oxygen demand	412	20			175																						
Flask test												4		6		14	459	140			120					9	47
Laboratory-scale activated sludge	33													2			118	101	26	31		15					
Warburg	754	27	13		205			11						5		5	15								4		
Other respirometric	51	2					3					1		43			3										
Laboratory-scale trickling filter																					7		8				

[a] Definitions of biodegradation test unit codes are given in Table A-1 in Appendix A.

TABLE 14. DISTRIBUTION OF TEST COMPOUND CONCENTRATION AS A FUNCTION OF TEST METHOD

	Test compound concentration (mg/L)								
	Not specified	0<1	1<5	5<10	10<20	20<50	50<100	100<500	>500
Biochemical oxygen demand	315		101	9	106	31	3	38	3
Flask test	39	42	73	149	211	84	30	237	10
Laboratory-scale activated sludge	3	18	51	10	21	16	31	166	10
Warburg	56		1	1	3	28	51	92	806
Other respirometric	5	5	2	4	15	31	3	6	32
Laboratory-scale trickling filter	0				5	10			

TABLE 15. DISTRIBUTION OF ACCLIMATION TIME AS A FUNCTION OF TEST METHOD

	Acclimation time (days)						
Test method	Not specified	Not acclimated	0<5	5<10	10<20	20<50	>50
Biochemical oxygen demand	350	179	10	2	24		
Flask test	45	306		109	207	96	6
Laboratory-scale activated sludge	41	2		16	49	68	12
Warburg	83	772	24	49	75	6	5
Other respirometric	43	20			32		2
Laboratory-scale trickling filter	15						

TABLE 16. DISTRIBUTION OF TEST TEMPERATURE AS A FUNCTION OF TEST METHOD

Test method	Acclimation time (days)					
	Not specified	15<20	20<25	25<30	30<35	>35
Biochemical oxygen demand	376	115	116			
Flask test	584	8	157	16	19	15
Laboratory-scale activated sludge	216	2	75	31		2
Warburg	178		724	35	79	23
Other respirometric	48		41	14		
Laboratory-scale trickling filter	15					

TABLE 17. DISTRIBUTION OF TEST MEDIUM AND INOCULUM AS A FUNCTION OF TEST METHOD

	Medium[a]					Inoculum[a]					
Test method	Not specified	NW	SW	SYN	WW	Not specified	Cult	NW	Soil	SW	WW
Biochemical oxygen demand	340	10		256	1	20	31	10	17		529
Flask test	4	59	17	699	20	288	88	59	16	7	341
Laboratory-scale activated sludge	40	9		229	48	43	2	9	31		241
Warburg	78			445	516	67	169		4		799
Other respirometric	6	6		75	16	5	5	1			92
Laboratory-scale trickling filter						15					

[a] Definitions of medium and inoculum codes are given in Table A-1 in Appendix A.

of test performance as they were of the properties of the tested substance. Therefore, any future use of these data in which biodegradability results from different literature sources are compared must recognize the influence of the test conditions on the biodegradability observed.

The alternative to this course of action was to attempt to correlate chemical structure and biodegradability for compounds that were tested by the same method and analytical protocol. This plan would essentially limit comparisons to data reported within a single reference or a very limited number of references. There are a number of studies in which a large number of compounds were tested by the same protocol. A listing of these studies is presented in Table 18. The most comprehensive of these studies is the static flask tests study of the priority pollutants conducted by the U.S. EPA.[33]

TABLE 18. REFERENCES REPORTING BIODEGRADABILITY DATA FOR MORE THAN FORTY COMPOUNDS

Document No.	Test method	Number of compounds
4004[a]	Warburg respirometer	75
4018	Warburg respirometer	44
4019	Warburg respirometer	76
4020	Warburg respirometer	139
4022	Shake or static flask	119
4037	Shake or static flask	102
5012	Warburg respirometer	49
8006	Biochemical oxygen demand	57

[a] Refers to references listed in Table A-2 in Appendix A.

ANALYSIS OF EXISTING SORPTION DATA

The largest data base regarding the sorption of organic chemicals onto biological solids is that obtained under an EPA contract to study the fate of priority pollutants in publicly owned treatment works.[6] Forty biological treatment plants receiving both municipal and industrial wastes were sampled at various points in the treatment process and the samples were then analyzed for the 129 priority pollutants. Data from only 20 of the plants were available during the time this search was being performed. Data from this study were analyzed to determine the degree of correlation between influent concentration of each of the compounds and compound loading on the primary sludge and correlations between compound concentration in secondary effluent samples and compound loading on secondary sludge samples. (Loading is defined as the concentration of substrate in the biomass on a dry weight basis.) Correlations were developed using only data points in which

TABLE 19. CORRELATION OF INFLUENT CONCENTRATION AND COMPOUND LOADING ON PRIMARY SLUDGE

Compound	Number of data points	Average influent concentration (µg/L)	Average compound loading on primary sludge (µg/g TSS)	Correlation coefficient (R)
1,1,1-Trichloroethane	7	99.7	1.00	0.22
1,1-Dichloroethane	8	2.75	2.57	0.11
1,2-Trans-dichloroethylene	6	8.83	25.3	0.22
Benzene	7	23.6	0.83	0.98
Bis(2-ethylhexyl)phthalate	11	31.0	82.7	0.10
Butyl benzyl phthalate	7	13.1	57.3	0.97
Di-n-butyl phthalate	9	12.0	4.60	0.08
Ethylbenzene	11	15.3	4.53	0.56
Methylene chloride	9	30.0	4.41	0.13
Naphthalene	5	4.6	5.37	0.99
Phenol	10	22.0	15.7	0.05
Tetrachloroethylene	6	42.8	1.83	0.03
Toluene	11	63.0	26.9	0.18
Trichloroethylene	9	75.5	3.57	0.37
				Average 0.36

measurable quantities of chemicals could be detected, i.e., analytical data reported as less than an analytical threshold or compound not detected were not used in the analysis. Correlations were performed only for compounds with more than five data points. The results of these analyses are presented in Tables 19 and 20.

TABLE 20. CORRELATION OF SECONDARY EFFLUENT CONCENTRATION AND COMPOUND LOADING ON SECONDARY SLUDGE

	Number of data points	Average effluent concentration (μg/L)	Average compound loading on sludge (mg/g)	Correlation coefficient (R)
Bis(2-ethylhexyl)phthalate	9	27.7	205.0	0.40
Methylene chloride	6	24.1	10.4	0.26
Toluene	6	4.0	313.0	0.48

As shown by the value of the correlation coefficient in these two tables, in most cases there was very limited correlation between the concentrations in the liquid phase and the loadings on the sludge.

Freundlich sorption isotherms developed from these data were poor because aqueous phase concentrations were generally in a narrow range of concentrations, while isotherms were normally developed with concentrations ranged over several orders of magnitude. Difference in sludge characteristics at different plants, such as variation in percent volatile solids, may have also accounted for some of the variability in loadings. Small differences in analytical values at the low concentrations frequently measured in the sludge phase may have yielded a large percentage variation in the calculated sludge loading values. Findings from this report (Sections 8 and 10) suggest that saturation may occur at critical concentrations that differ between compounds. If true, determination of the critical saturation concentration is needed for data interpretation.

ANALYSIS OF EXISTING STRIPPING DATA

Because the experimental procedures varied so widely and the number of compounds studied was so small, no statistical analysis of existing stripping data was undertaken. A review of the database on stripping from activated sludge systems is presented in Section 6.

SECTION 4

SELECTION OF COMPOUNDS FOR STUDY

SUMMARY

Because this study was planned to cover such a broad categorization of mechanisms (stripping, sorption on biomass, and bio-oxidation) with a limited budget and schedule, the selection of test compounds for study was a critical factor in the design of the study. Selection criteria were developed and applied to a large list of aromatic compounds, resulting in a limited set of compounds that potentially represented a wide range of variation in each removal mechanism. It was hoped that this selection process resulted in a group of study compounds that adequately tested the limits of each experimental protocol and allowed tentative determination of the applicability of the protocols to the larger universe of organic compounds. It is understood that more testing is necessary for actual application of any of these protocols to new and different compounds.

BACKGROUND

From the inception of this study, it was recognized that it was not possible, within project constraints, to develop and prove the feasibility of experimental and mathematical predictive fate methods for a large number of compounds. Instead, our emphasis has been to carefully select compounds that should be widely representative and would test the limits of experimental methods and to study these substrates leading to the development of experimental and mathematical protocols. Careful selection of differing compounds would suggest that the limits to subsequently developed methodology would be broad. Work could be identified to further test and refine each protocol and to more exactly establish its applicability to the universe of organic compounds.[36] With this philosophy, the selection of the original, relatively small set of compounds for study became quite important.

Although the original work directive for this work assignment included study of several groups of priority pollutants, our work plan was based on study of compounds from only one group - aromatic compounds. The rationale for this was based on three factors. First, the grouping "aromatics" included several of the subgroupings of interest to EPA. Second, an ultimate goal of this study was to determine if fate prediction of organic chemicals using structure-treatability relationships was feasible. Since little suitable data existed on which to base structure-fate relationships, the data generated herein were to be used. Substituted aromatics are a convenient and important group of compounds to study since an information base on chemical reactivity of various substituents on the ring has already been developed by organic chemists.

Finally, preliminary work indicated that the bio-oxidation mechanism was to be the most difficult on which to base both experimental and mathematical fate predictors. Catabolic degradation of aromatics as a group is thought to proceed by a few common pathways, first, by oxidative steps to catechol, then through a few similar dioxygenase ring cleavage steps. Further description of these pathways is presented in Section 9. Study of the biological degradation of aromatics as a class was hoped to eliminate some of the variability expected if compounds with no common pathways were selected for study.

EPA also requested the inclusion of a real waste stream as the basic wastewater into which the selected compounds were added. The EPA requested that a few of the compounds actually in the waste selected be studied. A more complete discussion of the waste stream is presented in Section 5.

METHODS

The selection process was a two-step process. First, a preliminary list of compounds was prepared that included 60 aromatic compounds and isomers. This list is presented in Table 21. This list includes both aromatic compounds potentially present in foul condensate waste streams as well as other aromatics representative of a variety of structural configurations. Several references were utilized to determine the possible aromatics present in foul condensate.[37,38] These data only identified specific aromatics in the total plant effluent. This would include contributions from the foul condensate as well as other sources. Since foul condensate typically comprises about 5% of the total wastewater from a pulp and paper facility, the analysis of reference data was used only for range-finding estimation. Compounds potentially present in the total wastewater are shown in Table 22. A priority pollutant screening test substantiated that only guaiacol was found at approximately 1 mg/L concentrations in the foul condensate.

Compounds on the long list were surveyed according to the following criteria in order to generate a "short list."

1. High (low) tendency to strip from wastewater
2. High (low) tendency to sorb onto biomass
3. High (low) tendency to bio-oxidize
4. Ease of conventional analysis
5. Cost and availability of radiolabeled compounds
6. Safety requirements
7. Member of EPA specified groupings

TABLE 21. ORIGINAL LIST OF AROMATICS CONSIDERED AS CANDIDATES FOR STUDY

Benzene
Toluene
o-Xylene
m-Xylene
p-Xylene
Hemimellitene
Pseudocumene
Mesitylene
Prehnitene
Isodurene
Durene
Pentamethylbenzene
Hexamethylbenzene
Ethylbenzene
n-Propylbenzene
Cumene
n-Butylbenzene
Isobutylbenzene
sec-Butylbenzene
tert-Butylbenzene
p-Cymene
Diphenylmethane
Triphenylmethane
1,2-Diphenylethane
Styrene
Chlorobenzene
Dichlorobenzene
Trichlorobenzene
Tetrachlorobenzene
Pentachlorobenzene
Hexachlorobenzene
Aniline
Methoxybenzene (Anisole)
Ethoxybenzene (ethyl phenyl ether)
Nitrobenzene
Dinitrobenzene
Benzonitrile
Benzoic acid
Benzene sulfonic acid
Benzaldehyde
Benzyl chloride
Phenol
o-,m-,p-Chlorophenol
o-,m-,p-Cresol
Catechol
Resorcinol
Hydroquinone
m-,p-Hydroxybenzoic acid
Salicylic acid
Chlorophenol
Dichlorophenols
Trichlorophenols
Tetrachlorophenol
Pentachlorophenol
o-,m-,p-Nitrophenol
o-Methoxyphenol
Biphenyl
Chlorinated biphenyl

TABLE 22. AROMATIC COMPOUNDS POTENTIALLY PRESENT IN PULP AND PAPER WASTEWATERS[a]

- Phenol
- 2,4-Dichlorophenol
- 2,4,6-Trichlorophenol
- Pentachlorophenol
- Benzene
- Toluene
- Xylene
- Ethylbenzene
- Guaiacol (o-Methoxyphenol)

[a] References 37 and 38.

The tendency to strip was estimated from an equilibrium case calculation that assumed the compound achieves its equilibrium concentration in the aeration basin off-gas based on calculated Henry's law constants. This method is discussed further in Section 6.

Relative tendency to sorb was calculated based on an assumption that the compound would sorb on biomass proportional to its octanol-water partition coefficient (K_{OW}). Worst-case calculations were performed using this assumption and were based on a model biological plant with varying levels of biomass. This method is described in Section 8. Values for vapor pressure, water solubility (to calculate Henry's law constants) and log K_{OW} were obtained from the ISHOW computer date base[39] and other standard property handbooks.[40,41]

Relative bio-oxidation tendency proved more elusive to estimate. A first attempt was made to compile and summarize data based on known or assumed degradation pathways. Microbiological professionals then speculated on the relative bio-oxidation rates of the various compounds. Finally, two experimental references were used to rank the compounds.[32,42] At times, these references disagreed in terms of how a particular compound should be ranked and the initial ranking was regarded only as a range-finding estimate.

The ease of the conventional analysis was determined based on the judgment of IT Corporation's professional analytical chemists.

The availability and cost of ^{14}C radiolabeled compounds was determined by obtaining estimates from Amersham, Inc., Pathfinder, Inc., and New England Nuclear, Inc. supply houses.

None of the compounds on the short list thus far was deemed to be an inappropriate safety hazard with the exception of benzene. It was decided to study toluene instead of benzene because of benzene-related health risks and burdensome extra record keeping requirements then in effect.

All compounds on the short list were of interest to EPA in one of their initial groupings.

RESULTS

The short list of compounds initially targeted for study in decreasing order of stripping, sorption, and bio-oxidation importance follows:

- Inclusion in Stripping Study
  - Toluene
  - 1,4-Dichlorobenzene
  - Phenol

- Inclusion in Sorption Study
  - Hexachlorobenzene
  - Pentachlorophenol*
  - 1,4-Dichlorobenzene*
  - Toluene
  - Aniline
  - Phenol*

*Priority - to be studied first; subsequent compounds tentative.

- Inclusion in Bio-oxidation and LAS Study
  - Aniline
  - Phenol
  - Toluene
  - Chlorobenzene
  - 1,4-Dichlorobenzene
  - 2,4-Dichlorophenol

The compound in the wastes, guaiacol, was dropped because of the analytical complexities, the low level in the waste, and the unavailability of ^{14}C-labeled guaiacol.

Problems were experienced in the implementation of all experimental phases requiring modification of the list of compounds studied. In stripping experiments, the time required in order to strip significant levels of phenol approached one week per experiment. This was inconsistent with the available budget so a substitute compound was sought for the low stripping compound. A suitable aromatic that had a Henry's law constant between phenol and 1,4-dichlorobenzene and that met our analytical requirements could not be identified. Methyl ethyl ketone was chosen because it fell into the needed stripping niche and was easily analyzed. Because of extensive unanticipated analytical development activities to measure specific compounds in the aqueous phase and biomass during sorption studies, only the three priority compounds - phenol, 1,4-dichlorobenzene, and pentachlorophenol - were studied in the sorption experiments.

Difficulty in the bio-oxidation experiments was experienced related to the inability to determine the population fraction of biomass microorganisms growing on chlorobenzene, 1,4-dichlorobenzene, and 2,4-dichlorophenol in mixed cultures. For this reason, emphasis was placed on extensive study of phenol, aniline, and toluene. Lab-scale experiments were run on phenol and toluene, and aniline was substituted for 1,4-dichlorobenzene late in the program. A special pentachlorophenol run was implemented in the LAS reactors with the intent of minimizing bio-oxidation and verifying the sorption predictor developed in Section 8.

The final list of compounds studied in this project was:

- Stripping
    - Toluene
    - 1,4-Dichlorobenzene
    - Methyl ethyl ketone
    - Phenol

- Sorption
    - Phenol
    - 1,4-Dichlorobenzene
    - Pentachlorophenol

- Bio-oxidation
    - Phenol
    - Toluene
    - Aniline
    - 2,4-Dichlorophenol (very limited testing)

- LAS
    - Phenol
    - Toluene
    - Aniline
    - Pentachlorophenol (biological rates minimized)

SECTION 5

SEED SLUDGE AND WASTEWATER FEED ACQUISITION AND HANDLING

BACKGROUND

Early in this study, a literature survey was implemented to review the existing data base on bio-oxidation rates of specific organic compounds in activated sludge systems. This analysis was discussed in Section 3. One of the findings of this effort was that much of the variation in biological assessment tests may be related to the type of inoculum used and its acclimation to the compound of interest.

In an attempt to reduce this variability in this study, a biomass was cultured over many months under carefully controlled conditions. This process is described below.

One of the requirements the EPA imposed on this study was to work with a real waste stream whenever possible. That is, compounds to be tested should be added to a wastewater arising from an actual industrial process. Synthetic waste streams were to be avoided.

IT Corporation, in a related work assignment (68-03-3027, WA2), had just completed an effort to identify an industrial source with wastewater and a treatment plant on which to base a full-scale material balance study. After expenditure of several man-months of effort, we were unable to locate a plant with a suitable wastewater (time invariant composition of waste containing a spectrum of biodegradable, strippable, and sorbable organic compounds) and also willing to cooperate with IT and EPA. Since this study required a similar waste in relatively large volumes, it was immediately clear that no suitable waste was available near the IT and UT Knoxville, Tennessee laboratories.

For these reasons, a waste stream possessing one of the attributes needed (time based uniformity) was selected. Pulp and paper mill foul condensate is a distillate of the liquor from the pulping process and is relatively uniform in composition over time. The existence of a cooperative source of this wastewater within 100 miles of the IT Corporation Knoxville facility was fortuitous. Since organic compounds were to be added to this wastewater and since the focus of this study was to determine methods to predict fates, not to determine the treatability of a specific industrial waste stream, the use of a foul condensate stream was proposed to and accepted by EPA.

SLUDGE ACQUISITION AND MAINTENANCE

The purpose of this portion of the experimental program was to develop and maintain a culture of microorganisms for use in treatability studies to be performed at IT Corporation and University of Tennessee, Knoxville. Use of a biological culture derived from the same source was expected to minimize experimental errors associated with relative differences in the types of organisms present in the sludge inoculum.

The activated sludge culture used for seeding the lab-scale activated sludge system was obtained from an activated sludge process operated by the Procter and Gamble Company at a sulfite process paper mill at Mehoopany, Pennsylvania. A 28-liter sample of return secondary activated sludge was taken and placed in 4-liter polyethylene containers with each container filled approximately 1/2 full. The containers were packed into coolers with approximately 22 Kg of ice per cooler. Samples were shipped to IT in Knoxville via air freight on October 10, 1982.

The sludge was split equally among three open-topped 208-liter (55-gal) initial acclimation reactors (IARs) or polyethylene lined steel drums. Each IAR was operated at a liquid level of 140 liters. The drums were initially prepared by loading each with 140 liters of tap water and aerating for 48 hours before the sludge inoculum was introduced. The experimental objective for each IAR is shown in Table 23.

TABLE 23. EXPERIMENTAL OBJECTIVES FOR INITIAL ACCLIMATION REACTORS (IARs)

Acclimation reactor no.	Feed	Experimental objective
1	Foul condensate, nutrients, methanol, and organic compounds	Acclimation of biomass to aniline, phenol, 2,4-dichlorobenzene, 1,4-dichlorobenzene, chlorobenzene, toluene
2	Foul condensate, nutrients	Control for reactor No. 1
3	Same as No. 2	Growth of biomass to be irradiated for sorption and stripping experiments

The reactors were fed with foul condensate, supplemented with a buffer solution, inorganic nutrients, and methanol. After approximately 8 weeks of acclimation to this feedstock, the feed to IAR-1 was spiked with six organic compounds. A buffer solution of K_2HPO_4 and KH_2PO_4 was added to maintain the system pH between 6.5 and 7.5. The quantity of phosphate buffers varied, depending upon the chemical composition of each batch of wastewater and the operating conditions of the individual biological reactors.

Ammonium nitrate was added to the foul condensate to maintain a 5:1 weight ratio of TOC:NH_3-N. Adequate phosphorus was available in the buffer solution to maintain a 25:1 ratio of TOC:P. In addition to nitrogen and phosphorus, other inorganic nutrients added to the foul condensate are listed in Table 24.

TABLE 24. NUTRIENT/BUFFER SOLUTION

Compound	Concentration in foul condensate feed (mg/L)
$CaCl_2$	100.0
NaCl	100.0
$MgSO_4$-H_2O	200.0
$FeCl_2$	5.0
NH_4NO_3	Varied depending on TOC
K_2HPO_4	Varied depending on system pH
KH_2PO_4	Varied depending on system pH

Analysis of foul condensate samples indicated that a significant percentage of the TOC was composed of methanol. ACS grade methanol was added to the wastewater to supplement the organic concentration. The methanol provided additional food for the growing microorganisms and reduced the volume of foul condensate used.

Biomass in initial acclimation reactor No. 1 was gradually acclimated to the first six organic compounds shown in the schedule in Table 25. The organic spike solution was prepared in 4 liters of deionized water which was continuously fed to the reactor over a 24-hour period.

Each of the acclimation reactors was operated as a fill-and-draw system. A mean cell residence time (MCRT) of approximately 5 days was maintained by removing 28 liters of mixed liquor from each reactor daily and replacing it with 28 liters of the foul condensate/nutrient mixture.

On February 23, 1983, the three 208-liter acclimation reactors were shut down. Sludge from IAR-1 was used to seed four 50-liter polyethylene acclimation and seed reactors (ASR). These ASRs were maintained to provide seed culture for the lab-scale activated sludge units which were used for fate studies for specific organic compounds. The ASRs were operated at liquid volume of 25 liters. These acclimation systems were operated as fill-and-draw batch reactors at mean cell residence times (MCRT) of 5, 7.5, 10, and 12.5 days, respectively. The acclimation schedule which was followed for both IARs and ASRs is shown in Table 25. (IARs were used until 2/26/83 and ASRs were used thereafter.)

TABLE 25. SCHEDULE FOR ACCLIMATION OF BIOMASS TO TEST COMPOUNDS[a]

Start Date	End Date	Concentration in Feed							
		Aniline (mg/L)	Phenol (mg/L)	Toluene (mg/L)	2.4-Dichlorophenol (mg/L)	1.4-Dichlorobenzene (mg/L)	Chlorobenzene (mg/L)	Pentachlorophenol (mg/L)	Methanol (mg/L)
12/01/82	12/13/82	3	3	3	3	3	3	0	1,600
12/14/82	12/15/82	6	6	6	6	6	6	0	3,000
12/16/82	12/18,82	40	40	40	12	12	12	0	2,500
12/19/82	12/22/82	50	50	50	14	14	14	0	2,500
12/23/82	12/26/82	70	70	70	16	16	16	0	2,500
12/27/82	12/30/82	100	100	100	18	18	18	0	2,500
12/31/82	01/03/83	150	150	150	20	20	20	0	2,500
01/04/83	02/26/83	200	200	200	20	20	20	0	2,500
02/27/83	03/07/83	0	200	200	20	20	0	0	0
03/08/83	06/28/83	0	135	135	13.5	13.5	0	0	0
06/29/83	07/08/83	50	0	0	0	0	0	0	0
07/09/83	07/11/83	100	0	0	0	0	0	0	0
07/12/83	07/26/83	100	0	0	0	0	0	0	0
07/27/83	08/04/83	100	0	0	0	0	0	5	0
08/05/83	08/16/83	0	0	0	0	0	0	5	0

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[a] Acclimation was carried out in 208 liter initial acclimation reactors (IARs) from 12/01/82 until 02/26/83. After that date, acclimation was carried out in 50 liter acclimation and seed reactors (ASRs). Actual date when biomass was used to seed LAS reactors is given in Table 84. Chemical concentrations were initially built up gradually, then eliminated as the LAS chemicals to be studied evolved.

Operating procedures for ASRs consisted of daily wasting of 1/MCRT (mean cell residence time) of the reactor mixed liquor, turning off the air supply and letting the sludge settle, and wasting enough of the supernatant so that the total volume wasted per day was equivalent to 10 liters. Each reactor was fed 10 liters of foul condensate supplemented with the same inorganic nutrients which had been used to maintain the IARs. Methanol supplementation to the feed was discontinued when the large IARs were shut down on February 26, 1983.

Feed preparation consisted of adding the organic spike compounds to 40 liters of foul condensate and mixing for 24 hours in a sealed 80-liter stainless steel drum.

WASTEWATER FEED ACQUISITION AND HANDLING

The wastewater feed used in this study was obtained from a foul condensate stream from a kraft process pulp and paper mill operated by the Bowater Pulp and Paper Company at Calhoun, Tennessee. The wastewater was a composite condensate stream from a six-effect evaporator equipped with contact condensers. Cooling water for the condensers is obtained from the Hiawassee River. The foul condensate/condenser water is combined and piped to a storage tank. Intermediate storage yielded a wastewater which was relatively uniform for all wastewater collected on a given day with moderate variation in chemical composition between batches collected on different days. A key guideline the EPA established at the beginning of this study was that the reactor feedstock must come from an operating industrial process. Adherence to this guideline involved accepting a substantial amount of variation in the chemical composition of wastewater samples which were collected at different times. During the laboratory-scale activated sludge study, experiments for each compound were conducted with foul condensate feedstock samples collected on the same date.

The major exception to the normally moderate variation in wastewater quality occurred with a wastewater sample collected on February 17, 1983. During the time that samples were being taken from the storage tank, condenser bottoms as well as condenser overheads were being pumped into the storage tank. The last samples collected on this day had a significantly higher TOC concentration than the first samples.

Foul condensate was collected in both 56.8-liter (15-gal) clean polypropylene carboys and 55-gal clean stainless steel drums. The wastewater was transported to the IT laboratory by truck. Wastewater was stored in a cooler at approximately 4°C until it was prepared as feed to a biological reactor.

Grab samples from each shipment of foul condensate were analyzed for BOD_5, COD, and selected other conventional parameters. The results of these analyses are presented in Table 26.

Samples collected on 8/17/83 were analyzed for priority pollutants by gas chromatography-mass spectrometry (GCMS). No priority pollutants were

TABLE 26. FOUL CONDENSATE ANALYTICAL RESULTS

Sample Collection Date	Sample Analysis Date	TOC (mg/L)	BOD_5 (mg/L)	COD (mg/L)	NH_3 (mg/L)	Total Phosphorus (mg/L)
08/16/82	08/16/82	300	542	1090		
08/11/82						
10/19/82						
11/02/82	11/02/82		1100	1300		
11/19/82	11/19/82		1010	1000	22	0.17
12/10/82	12/10/82	486	1200	1860	20	0.10
01/07/83			800	1400		
02/17/83	02/17/83		540	1000		
	02/17/83		1900	4100		
03/16/83	03/16/83		700	900		
05/12/83	05/19/83	491				
	05/31/83		820			
	06/03/83		730			
	06/08/83		700			
	06/22/83		830			
06/23/83	06/25/83		769			
	06/29/83		400			
	07/12/83		550			
	07/13/83	387				
07/27/83	07/27/83					
	08/05/83		700			
	08/12/83		460			
	08/23/83	385				
	09/02/83		510			

detected. Analytical results for both nonspecific parameters (BOD_5, COD) and some specific organic compounds in this sample are presented in Table 27. Data in this table indicate that the theoretical oxygen demand for the oxidation of methanol is approximately 64% of the BOD_5 value and that methanol theoretically represents approximately 30% of the TOC.

TABLE 27. ANALYTICAL RESULTS FOR FOUL CONDENSATE SAMPLE COLLECTED ON 8-17-82

Parameter	Concentration (mg/L)	Theoretical oxygen demand (mg/L)	Theoretical total organic carbon (mg/L)
BOD_5	542		
COD	1090		
TOC	300		
Methanol	230	345	86
Ethanol	<15	<31	<8
Acetic acid	<100	<10	<40

A metals screening analysis of a foul condensate sample collected on 12/10/82 was conducted to check for potentially toxic concentrations of heavy metals. Analysis was conducted using an inductively coupled argon plasma (ICAP) instrument. Data from this analysis are included in Table 28. Concentrations of heavy metals were generally in the low ppb range, indicating that concentrations of heavy metals present were generally not a concern from a toxicity standpoint.

TABLE 28. METALS ANALYSIS OF FOUL CONDENSATE COLLECTED ON 12-10-82

Metal	Concentration (mg/L)
Aluminum	<0.01
Antimony	<0.05
Arsenic	<0.2
Barium	0.005
Beryllium	0.003
Boron	0.02
Cadmium	<0.002
Calcium	2.8
Chromium	<0.02
Cobalt	<0.01
Copper	<0.01
Iron	0.036
Lead	<0.05
Lithium	<0.03
Magnesium	0.771
Manganese	0.030
Molybdenum	<0.02
Nickel	<0.02
Selenium	<0.2
Silicon	1.00
Sodium	72.
Strontium	0.0103
Silver	<0.01
Thallium	<0.3
Tin	<0.2
Titanium	<0.003
Vanadium	<0.01
Zinc	<0.01

SECTION 6

DEVELOPMENT OF A STRIPPING PREDICTIVE FATE METHOD

SUMMARY

This study was performed to develop an approach to measure and ultimately predict the stripping of organic chemicals from activated sludge processes, given physical property information on the chemical and design data on the treatment plant. This approach endeavors to develop experimental and mathematical stripping protocols and is called a stripping predictive fate method (PFM).

This method offers a simpler approach for estimating stripping rates in pure water and wastewater than other approaches based on the two-film theory of mass transfer. This method incorporates the Henry's law constant and the air flow-to-liquid volume ratio to directly yield the first-order stripping rate constant for pure water systems. Equations predicting the stripping rate constant from the compound Henry's law constant follow the form of $K_a{}^{st}a \cdot V/Q_{air} = 3.71 \times 10^{-3} (H_c)^{1.045}$ for a pure water system. When the dimensionless Henry's law constant is used, the dimensionless equation, $K_a{}^{st}a \cdot V/Q_{air} = H_i$, is developed. This is strong evidence that stripping is an equilibrium process in coarse-bubble diffusion systems.

The presence of surfactants, salts, oils, and nonviable biomass was found to vary the stripping rate, but in no case was the effect more than ±50% when compared with pure water (distilled or tap water). The variations seem to be the strongest for the compounds with higher Henry's law constants.

The values of the stripping rate constant, $K_a{}^{st}a$, predicted from the model of this study seem to agree reasonably well with previously reported values. This suggests the possibility of application beyond the compounds studied herein.

The stripping predictive fate method was tested in continuous lab-scale activated sludge units. The measured toluene stripping rate was found to be consistent with the predicted toluene stripping rate constant. This further substantiates that the stripping predictive fate method may be useful for prediction of compound stripping in continuous "real world" systems.

INTRODUCTION

Wastewater treatment plants often receive volatile compounds in their influent from industrial discharges or from other sources. The removal of the refractory pollutants and chemicals from water and introduction to the atmosphere by the stripping mechanism may be an important environmental pathway in wastewater treatment facilities.

Stripping is distinguished in this report from volatilization by the mode of gas-liquid contact. Stripping is the process of gas/liquid mass transfer in systems with dispersed gas or liquid phases. Volatilization occurs at the major surface between gas and liquid phases. Stripping processes normally have a much greater interfacial area and are likely to occur in a more turbulent hydraulic regime than volatilization processes.

The stripping of chemicals dissolved in water can be expressed as a first order kinetic rate equation:

$$-r^{st} = -\frac{dC_a^{st}}{dt} = K_a^{st}a\ C_a \qquad \text{Eqn. 17}$$

where:

$-r^{st}$ = the rate or rate equation for stripping of substrate from the aqueous compartment into the gas-vapor compartment (mg L^{-1} hr^{-1}),

$-\frac{dC_a^{st}}{dt}$ = instantaneous rate of change of substrate concentration in the aqueous compartment related only to stripping of substrate into the gas-vapor compartment (mg L^{-1} hr^{-1}),

$K_a^{st}a$ = substrate stripping rate constant based on aqueous phase disappearance (hr^{-1}),

a = interfacial area (m^2), and

C_a = substrate concentration in the aqueous compartment (mg/L).

In applying the two-film mass transfer model for volatilization processes, Liss and Slater[43] and Mackay and Leinonen[8] have shown that:

$$K_a^{st}a = \frac{a}{V}\left[\frac{1}{k_l^{st}} + \frac{RT}{H_c\ k_g^{st}}\right]^{-1} \qquad \text{Eqn. 18}$$

where:

k_l^{st} = liquid film mass transfer coefficient of the substrate (m hr^{-1}),

k_g^{st} = gas film mass transfer coefficient of the substrate (m hr^{-1}),

a = interfacial area (m^2),
V = total of the fluid volume (m^3),
R = universal gas constant (62.4 torr L/g-mole °K),
T = absolute temperature (°K), and
H_c = Henry's law constant (torr L/g-mole).

Similar equations can be applied to oxygen transfer:

$$K_a^o a = \frac{a}{V}\left[\frac{1}{k_l^o} + \frac{RT}{H_c k_g^o}\right]^{-1} \qquad \text{Eqn. 19}$$

where:

$K_a^o a = K_L a$ = oxygen reaeration rate constant (hr^{-1}), and

k_l^o, k_g^o = oxygen liquid and gas film mass transfer coefficient, respectively.

For compounds with moderate to high Henry's law constants, k_g^o is often negligible. Environmental engineering convention uses the symbol $K_L a$ to describe oxygen transfer. This study will also use $K_L a$ for oxygen transfer.

Smith and other authors[44,45,46] have shown that the volatilization rate of chemicals can be controlled by mass transfer resistance in the liquid phase (for high volatility compounds), gas phase (for low volatility compounds) or a combination of both (transition volatility compounds), depending on the value of H_c. The volatilization rate constant for high volatility compounds, with values of H_c >3500 torr L/g-mole (0.0044 atm m^3 g-mole^{-1}) at 25°C is. . .

$$\frac{K_a^{st} a}{K_L a} = \frac{k_l^{st}}{k_l^o} = \left[\frac{D_l^a}{D_l^o}\right]^m = \text{constant} \qquad \text{Eqn. 20}$$

where:

m = between 0.5 and 1.0, and

D_l^a, D_l^o = molecular diffusion coefficient of substrate and oxygen in the liquid phase, respectively.

Similar relationships may be derived for transition volatility compounds, with H_c between 10 and 3500 torr L/g-mole and low volatility compounds, with H_c <10 torr L/g-mole. The volatilization rate constant for transition volatility compounds is. . .

$$K_a^{st}a = \frac{a}{V}\left[\frac{1}{k_l^{st}} + \frac{RT}{H_c k_g^{st}}\right]^{-1} = \frac{a}{V}\left[\frac{1}{k_l{}^{o}B} + \frac{RT}{H_c k_g{}^{w}F}\right]^{-1} \qquad \text{Eqn. 21}$$

with

$$\frac{k_g^{st}}{k_g^{w}} = \left[\frac{D_g^{a}}{D_g^{w}}\right]^{n}$$

$$B = \left[\frac{D_l^{a}}{D_l^{o}}\right]^{m} \qquad \text{Eqn. 22}$$

$$F = \left[\frac{D_g^{a}}{D_g^{w}}\right]^{n} \qquad \text{Eqn. 23}$$

where:

n = between 0.5 and 1.0,

k_g^{w} = gas phase mass transfer coefficient for water, and

D_g^{a}, D_g^{w} = molecular diffusion coefficient of chemical and water in the gas phase, respectively.

The volatilization rate constant for low volatility compounds is as follows:

$$K_a^{st}a = \frac{a}{V}\left[\frac{RT}{H_c\, k_g{}^{w}F}\right]^{-1} \qquad \text{Eqn. 24}$$

This approach has been tested and it has been shown that if the values of chemical diffusion coefficients are known, the values of the volatilization rate constant of these chemicals can be obtained for natural water systems.[45]

This study sought to determine suitable experimental methods for collecting stripping rate data, then tested the available mathematical predictive methods based on the two-film theory, to determine if they might be applied to biological treatment processes.

MATERIAL AND METHODS

Chemicals

Four compounds were chosen for the study of the stripping PFM. Phenol and methyl ethyl ketone were the low Henry's law constant compounds and

1,4-dichlorobenzene and toluene were the transition and high Henry's law compounds. Their physical properties are listed in Table 29.

The following chemicals were used in the stripping experiments:

- Toluene: J. T. Baker, Lot 116869 purified
- 1,4-Dichlorobenzene: Baker, Lot 137502
- Phenol: Fisher, Lot 782304 ACS
- MEK: Burdick & Jackson, Lot 8608
- Iso-octane: Fisher, Lot 790203, pesticide grade
- 1-octanol: Mallinckrodt, Lot 2F27-CE2063, organic reagent
- Triton-DF12: Rohm & Haas, Lot G-1528
- TAMOL-731: Rohm & Haas, Lot G-1588
- Marasperse N 22: Lignin Chemicals, Lot GN05L-1352
- DFOAM ST: Selig Chemical Company

Process Equipment

Figure 4 presents a diagram of the basic stripping apparatus.

Toluene Equipment --

A 6-ft high, 6-in. diameter glass column equipped with a coarse bubble diffuser was used to introduce the air. The coarse bubble diffuser was made from a 2½-in. 304 SS threaded pipe cap. The top of the cap was milled flat and eight 1/32-in. diameter holes were drilled evenly spaced around a 1-3/4-in. diameter concentric circle.

Air at about 12% constant relative humidity was fed into the system from the building air supply through a pressure reducer, a regulator, a pressure meter, a 2-ft by 2½-in. carbon filter, a pressure relief valve, an air rotameter, and a YSI model 91 dew point hygrometer. An air reading thermometer, and an inlet pressure reading manometer was attached to the feed air line.

Liquid samples were taken from the reactor through a ¼-in. OD 304 SS tube located in the middle of the column about 2½ ft from the bottom.

Test solution was pumped to the bottom of the column by a SATURN® Model SP-3000 Teflon® pump. Nitrogen was sparged into the bottom of the system at the diffuser level through a ¼-in. OD-304 SS tube entering from the top. Outlet pressure was measured with a manometer placed at the condenser inlet line.

The oxygen concentration was measured with a YSI model 54A dissolved oxygen meter with the probe hanging upside down in the middle of the column at the same level as the top of the liquid sampling line. Solution temperature was measured with a themocouple located about 3 in. below the liquid level.

Outlet air samples were taken from a sample port located on the condenser outlet line. Outlet air flow was checked by an outlet rotameter placed next to the sample port.

TABLE 29. PHYSICAL PROPERTIES OF THE COMPOUNDS STUDIED

	Toluene	1,4-Dichlorobenzene	Methyl ethyl ketone	Phenol
Molecular weight	92.1	147	72.1	94.1
Specific gravity	0.866	1.458	0.805	1.071
Boiling point (°C)	110.8	173.4	79.6	182.0
Vapor pressure[a] (mm Hg at 20°C)	22.0	0.6	77.5	0.2
Solubility[a] (mg/L at 20°C)	732.0	79.0	322,600	74,500
Henry's law constant[b]				
$\frac{(\mathrm{atm} - \mathrm{m}^3)}{(\mathrm{g\text{-}mole})}$	3643×10^{-6}	1469×10^{-6}	22.8×10^{-6}	0.33×10^{-6}
$\frac{(\mathrm{torr\ L})}{(\mathrm{g\text{-}mole})}$	2769	1116	17.3	0.25
Dimensionless[c]	0.151	0.061	9.4×10^{-4}	1.37×10^{-5}

[a] From "ISHOW" data base.[48]

[b] Calculated from Dilling.[47]

[c] Defined in Eqn. 25.

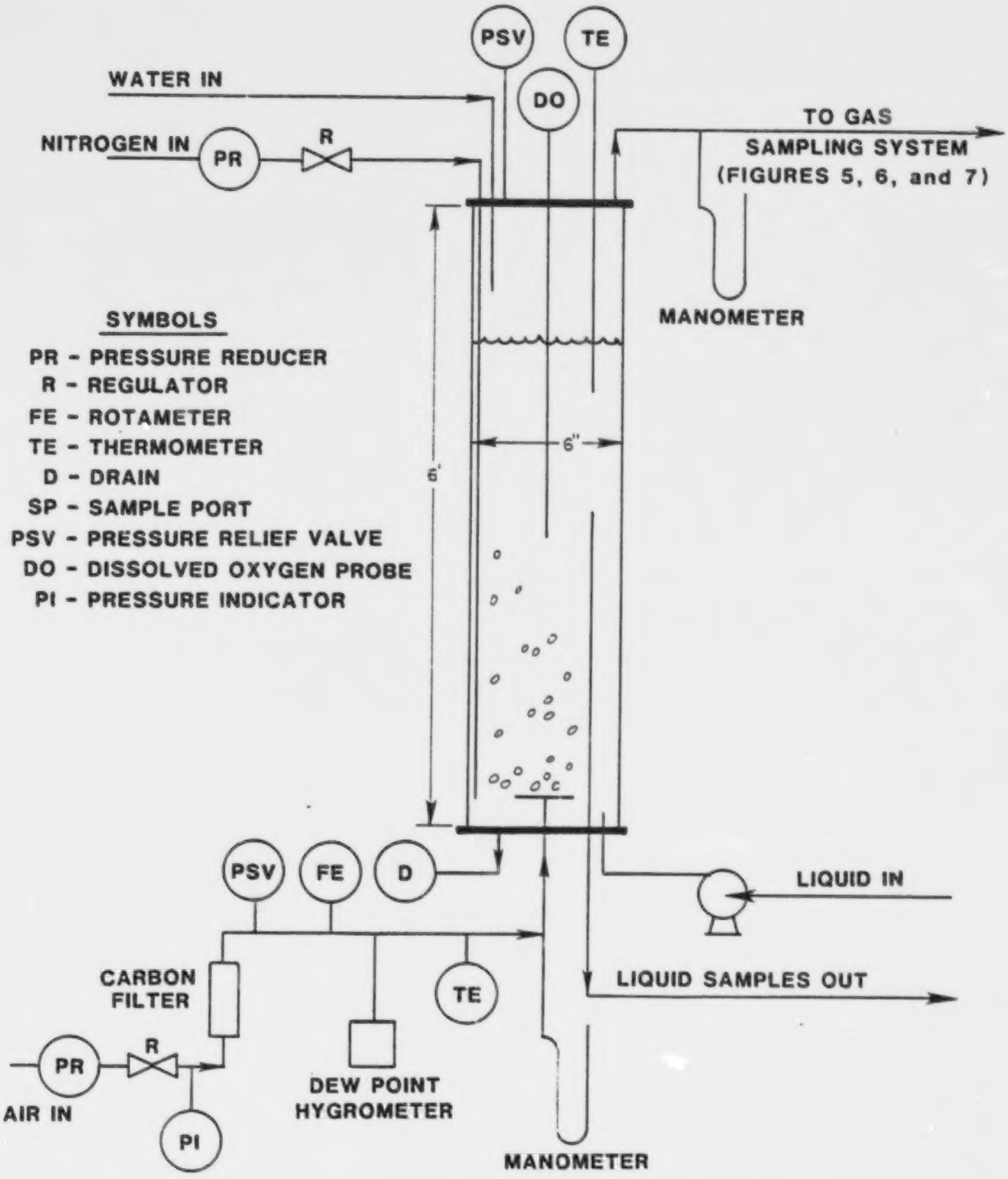

PSV
TE
DO
WATER IN
NITROGEN IN
PR
R
TO GAS SAMPLING SYSTEM (FIGURES 5, 6, and 7)
MANOMETER
SYMBOLS
PR - PRESSURE REDUCER
R - REGULATOR
FE - ROTAMETER
TE - THERMOMETER
D - DRAIN
SP - SAMPLE PORT
PSV - PRESSURE RELIEF VALVE
DO - DISSOLVED OXYGEN PROBE
PI - PRESSURE INDICATOR
6'
6"
PSV
FE
D
LIQUID IN
CARBON FILTER
TE
LIQUID SAMPLES OUT
PR
R
AIR IN
PI
DEW POINT HYGROMETER
MANOMETER

FIG. 4. APPARATUS USED IN THE STRIPPING EXPERIMENTS

In experiments ST-1 to ST-8, the equipment described above was used (Figs. 4 and 5). In experiments ST-9 to ST-19, five Tedlar® air bags were connected to the outlet line for the first 5 minutes of air sampling (as shown on Fig. 5). Surface tension was measured in all experiments by using a Cenco-Dunouy Model 70535 tensiometer. A bench-scale Model 7104-EHB 5/8-hp power mixer was used in an associated vessel to dissolve the chemicals into the solution.

Phenol and 1,4-Dichlorobenzene Equipment --
The equipment design was the same as for toluene experiments except that no air sample ports or outlet rotameters were used. Instead, a train of three 250 mL bubblers was added at the exit line as shown in Fig. 6; thermometers were also added at the inlet and outlet of the condenser.

Methyl Ethyl Ketone Equipment --
The equipment used was the same (see Fig. 7) as for the toluene experiments (ST-1 to ST-8), with the exception that air sample bags were not required.

Preliminary Calculations

The purpose of this section is to obtain Henry's law constants of these chemicals by applying Dilling's equation,[47] and to calculate the maximum amount of substrate stripped at equilibrium for a given set of operating conditions.

Calculation of Henry's Law Constant --
The Henry's law constant of the compounds studied was calculated by applying the following equations from Dilling.[47]

$$H_i = \frac{C_{air}}{C_{water}} = \frac{16.04\ p_o MW}{TS} \quad \text{and} \qquad \text{Eqn. 25}$$

$$H_c = H_i\ RT \quad \text{or}$$

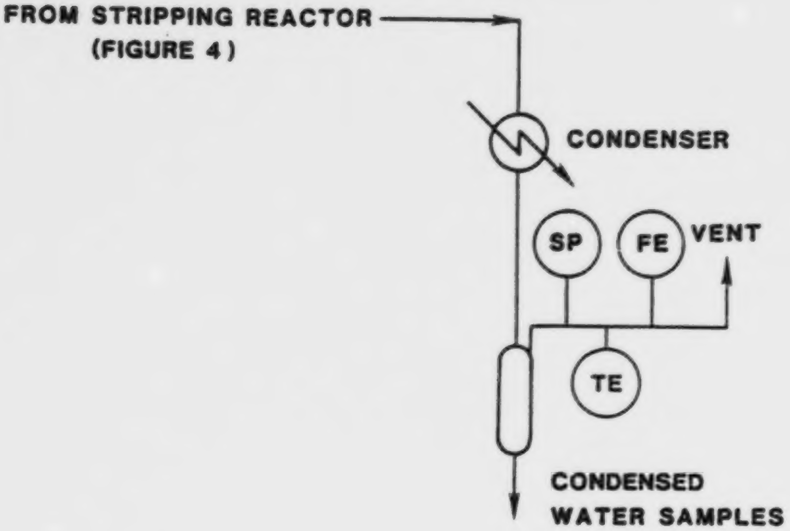
$$H_c = 13.16 \times 10^{-4}\ \frac{p_o MW}{S} \qquad \text{Eqn. 26}$$

where:

H_i = Henry's law constant (dimensionless concentration ratio, not mole fraction),
H_c = Henry's law constant (atm m^3/g-mole),
p_o = vapor pressure of the pure compound (mm Hg),
S = solubility of the compound in water (mg/L),
MW = gram-molecular weight of the compound,
T = temperature (°K), and
R = gas law constant = 8.2057×10^{-5} atm m^3/g-mole °K.

The values of S and p_o were obtained mostly from ISHOW data base.[48] The calculated values of H_c for the four compounds studied can be found in Table 29.

EXPERIMENT ST-1 TO ST-8


FROM STRIPPING REACTOR
(FIGURE 4)
CONDENSER
SP
FE
VENT
TE
CONDENSED
WATER SAMPLES

EXPERIMENT ST-9 TO ST-19

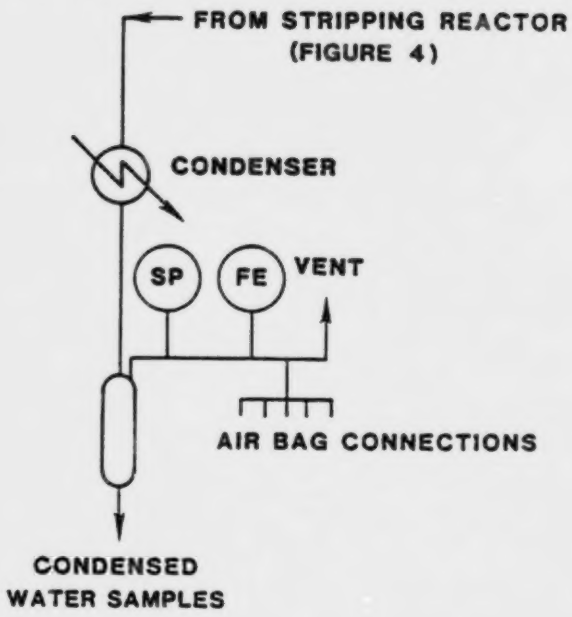

FROM STRIPPING REACTOR
(FIGURE 4)
CONDENSER
SP
FE
VENT
AIR BAG CONNECTIONS
CONDENSED
WATER SAMPLES

FIG. 5. GAS SAMPLING APPARATUS - TOLUENE EXPERIMENTS

EXPERIMENT ST-20 TO ST-32 AND ST-46, ST-47

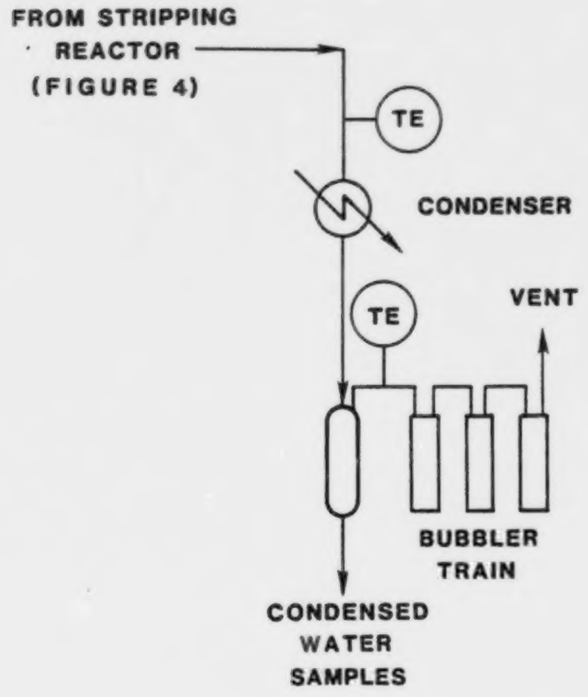
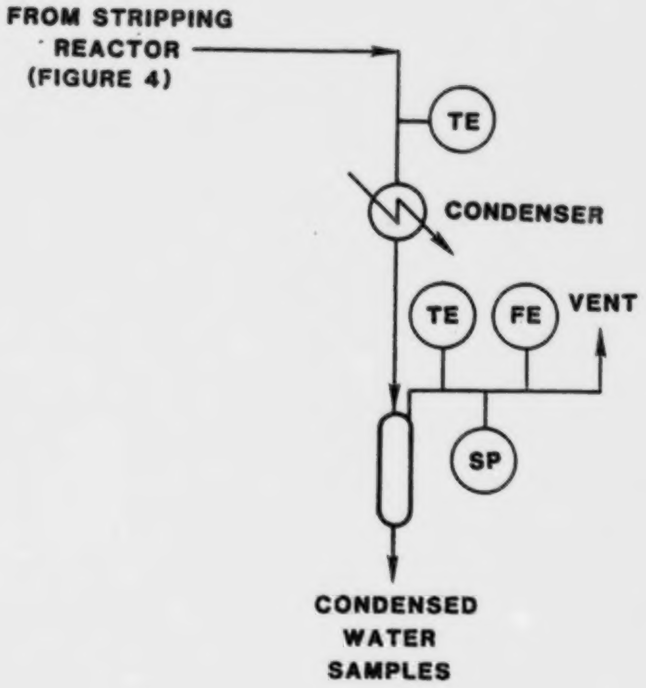

FROM STRIPPING REACTOR (FIGURE 4)
TE
CONDENSER
TE
VENT
BUBBLER TRAIN
CONDENSED WATER SAMPLES

FIG. 6. GAS SAMPLING APPARATUS - PHENOL AND 1,4-DICHLOROBENZENE EXPERIMENTS



EXPERIMENT ST-33 TO ST-45

FROM STRIPPING REACTOR (FIGURE 4)
TE
CONDENSER
TE
FE
VENT
SP
CONDENSED WATER SAMPLES

FIG. 7. GAS SAMPLING APPARATUS - METHYL ETHYL KETONE EXPERIMENTS

Worst-Case Equilibrium Stripping --

A worst-case equilibrium calculation of stripping potential was made as a function of Henry's law constants, assuming that equilibrium was achieved and no bio-oxidation or sorption was present. This was used to select the compounds for study (Section 4).

The material balance for a complete-mixed nonbiological system at equilibrium can be described as:

$$Q_{air}^{in} C_{air}^{in} + Q_a^{in} C_{ao} = Q_{air}^{out} C_{air} + Q_a^{out} C_a + Q_s^{out} C_s \qquad \text{Eqn. 27}$$

where:

Q_a^{in}, Q_a^{out} = inlet and outlet aqueous flow, respectively (L/min),

Q_{air}^{in}, Q_{air}^{out} = inlet and outlet air flow, respectively (L/min),

Q_s^{out} = outlet sludge flow (L/min),

C_{ao}, C_a, C_s = molar substrate in the inlet, outlet water, and sludge respectively (g-mole/L water), and

C_{air}^{in}, C_{air}^{out} = chemical concentration in the inlet and outlet air respectively (g-mole/L air).

If there is no substrate in either the sludge or the inlet air,

$$C_s^{out} = 0$$

$$C_{air}^{in} = 0$$

Also assuming . . .

$$Q_a^{in} = Q_a^{out} = Q_a \text{ and } Q_{air}^{in} = Q_{air}^{out} = Q_{air}$$

at equilibrium, the Henry's law constant $H_i = \frac{C_{air}}{C_a}$ (dimensionless), so Eqn. 27 then becomes:

$$Q_a C_{ao} = Q_{air} C_{air} + Q_a C_a \qquad \text{Eqn. 28}$$

or:

$$F^{st} = \frac{Q_{air}\ C_{air}}{Q_{ao}\ C_{ao}} = \frac{Q_{air}}{Q_{air} + (Q_a/H_i)} \qquad \text{Eqn. 29}$$

where F^{st} is the weight fraction of substrate stripped based on that feed to the system. Using an air flow rate of 2 L min^{-1}, a liquid flow rate of 11 L/24 hr, (0.0076 L min^{-1}); the maximum percentage of recovery of four represented compounds was calculated and listed in Table 30.

TABLE 30. MAXIMUM REMOVAL BY STRIPPING OF TOLUENE, 1,4-DICHLOROBENZENE, METHYL ETHYL KETONE, AND PHENOL

Q_{air} (L/min)	Q_a (L/min)	$\frac{Q_{air}}{Q_a}$	Maximum weight percent stripped			
			Toluene	1,4-DCB	MEK	Phenol
2	0.0076	263	97.5	94.1	20.0	0.36

Even though this may range an order-of-magnitude higher than a real system (in which bio-oxidation and stripping are at play) it establishes that compounds with Henry's law constants (H_c) less than 10 can only achieve levels of stripping 0.3 to 3.0% of the feed when the air to wastewater volume ratio is assumed to be 263. (See Section 11 for a more complete discussion of air flow and system volume effects on stripping.) Transition organics (compounds with Henry's law constants in torr L/g-mole between 10 and 2800) and high Henry's law organics (Henry's law constant greater than 2800) can result in worst-case stripping of between 20 and 98% of the organic feed at the same air to wastewater feed ratios.

Dispersion Test Experimental Procedure

The purpose of these preliminary experiments was to assure that the contents in the reactor were well mixed and uniform in composition throughout the reactor within the range of air flow rate chosen (2 L/min, 4 L/min, and 8 L/min). This characteristic is represented by the dimensionless reactor dispersion number, D/uL, which varies from zero for plug flow to infinity for completely mixed flow.[49] The variance of the concentration age distribution of the concentration profile, σ^2, can be expressed in the form of the following equations:

$$\sigma^2 = \frac{\sigma_t^2}{\bar{t}^2} = 2\left(\frac{D}{uL}\right) + 3\left(\frac{D}{uL}\right)^2 \qquad \text{Eqn. 30}$$

where:

$$\sigma_t^2 = \frac{\Sigma\, x_i^2\, f(x_i)}{\Sigma\, f(x_i)} - \left[\frac{\Sigma\, x_i\, f(x_i)}{\Sigma\, f(x_i)}\right]^2 \qquad \text{Eqn. 31}$$

$$\bar{t} = \frac{\Sigma\, t_i C_i \Delta t_i}{\Sigma\, C_i \Delta t_i} \qquad \text{Eqn. 32}$$

$$\Sigma\, f(x_i) = \Sigma\, C_i \Delta t_i \qquad \text{Eqn. 33}$$

$$\Sigma\, x_i\; f\,(x_i) = \Sigma\, t_i C_i \Delta t_i \qquad \text{Eqn. 34}$$

$$\Sigma\, x_i^2\; f(x_i) = \Sigma\, t_i^2 C_i \Delta t_i \qquad \text{Eqn. 35}$$

Units for the above equations are: C_i is the concentration of tracer input in mg/L, t is time in minutes, D/uL is the dimensionless dispersion number where D is the dispersion coefficient, u is the superficial liquid velocity, and L is the vessel length.

The 6-ft high by 6-in. ID glass column described earlier in the Material and Methods section served as the reactor; the YSI model 33 S.C.T. conductivity meter was used to measure the salinity of the solution.

Two methods were used to determine dispersion: one used the reactor as a batch reactor and the other used the reactor as a continuous reactor.

Fifty mL of 190,000 mg/L of salt (NaCl) solution was injected at the bottom of the batch reactor which contained about 26 L of water. The salinity of the water was detected by the probe hanging about 2 in. below water level (or 5 ft from the bottom of the reactor), and the concentration of the solution was recorded at regular intervals.

The same procedure used in the batch system was used in the continuous test except that water was pumped continuously to the bottom of the reactor at a flow rate of 3 L per minute, and the water outlet line was connected to the top of the reactor. A sample data calculation is presented in Appendix C.

Both tests showed high dispersion numbers, indicating that the reactor approximated the completely mixed case for the range of air flows studied.

Analytical Methods

Detailed methods for the analyses of toluene, 1,4-dichlorobenzene, methyl ethyl ketone, and phenol in this study are outlined in Appendix B. The methods were chosen or developed to provide valid analytical data for each compound and matrix tested. The majority of the samples were analyzed immediately after sampling to minimize losses. When necessary because of time and instrument constraints, a small percentage of samples were stored in a refrigerator for analyses on the following day.

Aqueous sample data are corrected for dilution when necessary and reported in units of mg/L. Gas sample data are reported in units of ppm in air (v/v in air).

Liquid Phase Sampling --

Aqueous samples were withdrawn from a 1/4-in. OD liquid sample port that sampled at a point approximately 2 1/2 ft above the bottom of the column. The sample line was flushed with 25 mL of aqueous sample and then a sample bottle was filled to overflowing and sealed with a Teflon-faced septum cap. Analytical samples were withdrawn with a microliter syringe through the septum. All aqueous phenol samples were covered with aluminum foil to minimize photolysis. Liquid phase sampling procedures were the same in all stripping experiments.

Vent Gas Sampling --

Vent gas samples containing toluene or methyl ethyl ketone were withdrawn with a valved, gas-tight syringe through an air sample port located at the condenser outlet air line. The syringe was flushed with ambient air several times and then inserted through the septum. The plunger was withdrawn to a volume approximately 50% greater than required and held for 20 seconds. The plunger was then depressed to the required volume and held for an additional 20 seconds. The valve was then closed and the syringe was withdrawn. For injection, the syringe was inserted into the instrument column septum, the valve was opened, and the plunger was depressed completely in a single smooth stroke. The syringe was then withdrawn with the plunger completely depressed.

Vent gas 1,4-dichlorobenzene samples were collected in fritted glass bubblers containing 150 mL of isooctane. The bubbler train was sampled at the end of each run by removing an aliquot of liquid from each bubbler and analyzing that aliquot. Total removal via the vent stream was calculated as the sum of the analyses from all of the bubblers in the train.

Vent gas analysis for phenol followed the same procedure as for 1,4-dichlorobenzene except that 0.1 N NaOH was used instead of isooctane.

Experimental Procedure

The main purpose of these experiments was to obtain the stripping rate constants and the oxygen reaeration rate constants of four different chemicals (toluene, 1,4-dichlorobenzene, methyl ethyl ketone, and phenol) at three air flow rates (2 L/min, 4 L/min, and 8 L/min). Sampling of the outlet air allowed the calculation of a material balance for the more volatile compounds. Experimental design also incorporated measurement of system-dependent variables so that the relationship between this experimental work and scaling to subsequent experiments and full-scale plants would be possible.

The general laboratory procedure for measuring stripping rate constants was to prepare a homogenous solution of chemical in another vessel at a concentration below its solubility limit, add the solution to the column, then analyze the aqueous concentration of the compound at regular intervals after air was introduced. Measurement of the oxygen reaeration rate constant was made for each experiment by initially purging the solution with nitrogen and then measuring the dissolved oxygen concentration as a function of time.

Toluene Experiments --

Experiments ST-1 -- This experiment determined the stripping rate of toluene in deionized water. Deionized water was deoxygenated by purging nitrogen to a 27-L polyethylene mixing carboy. Because toluene solubility was 732 mg/L, 12 mL of toluene was chosen to add to the water to make a starting solution of about 385 mg/L, which was well below toluene's solubility limit. The carboy was then hand mixed for about 15 min, and the solution was observed to be completely clear, indicating that all toluene was dissolved. The solution was then pumped into the reactor, and air was allowed to diffuse to the column at a preset air flow rate.

The dissolved oxygen concentration was read from the dissolved oxygen meter at 1 or 2 min intervals. Liquid phase analysis was performed by flushing about 25 mL of liquid out of the line before an actual sample was taken for analysis, the liquid samples were taken at intervals of 10 to 20 minutes apart.

Samples of the outlet air were taken for analysis at the same time as the liquid phase samples. About 100 mL of aqueous solution was also taken for surface tension measurement; three or four samples were required for each experiment. Liquid phase sampling procedures were the same in all experiments.

Experiment ST-2 -- This experiment determined the stripping rate of toluene in tap water. Tap water was deoxygenated by purging nitrogen to the mixing carboy. Twelve mL of toluene was added and stirred with the power mixer for 1 hr. Nitrogen was purged again to the solution for 15 min. The deoxygenated toluene solution was then pumped to the reactor, and air was allowed to diffuse to the column at a preset air flow rate.

Experiments ST-3 and ST-4 -- Since no significant difference was found between DI water and tap water, the term "pure water" will be used in this report to denote either DI water or tap water. These experiments determined the stripping rates of toluene in pure water.

The same procedure was applied in these two experiments as in experiment ST-2, except that toluene was added first to the mixing carboy, stirred with the power mixer for 30 min and purged with nitrogen for another 30 min, and then the deoxygenated solution was pumped to the reactor.

Experiment ST-5 -- This experiment determined the stripping rate of toluene in pure water. The same procedure was applied in this experiment as in experiments ST-3 and ST-4 except that after the solution was stirred in the mixing carboy for ½ hr, it was pumped to the reactor first and then was purged with nitrogen for 15 min.

Experiments ST-6, ST-7, and ST-8 -- These experiments determined the stripping rate of toluene in the presence of a Triton DF-12 solution. The same procedure was applied in these three experiments as was used in experiment ST-5, except that 2.8 g of surfactant Triton DF-12 was added. Twelve mL of toluene and 2.8 g of Triton DF-12 were added at the same time to 27 L of pure water in the mixing carboy. This was then stirred with the power mixer at a high speed for 30 min. The solution was pumped to the reactor, and then deoxygenated by nitrogen purging.

Experiments ST-12, ST-13, ST-17, and ST-18 -- These experiments determined the stripping rate of toluene in Triton DF-12 solution. Twelve mL of toluene was added to 27 L of pure water in the mixing carboy and was stirred with the power mixer at high speed. Nitrogen was purged to the mixing carboy for 10 min, while the pump recirculated the solution. A 2.8 g portion of Triton DF-12 was added to the mixing carboy and was stirred for 2 min; the solution was then pumped to the reactor and nitrogen was purged again to the solution until the dissolved oxygen concentration in the column was less than 0.5 ppm.

Differences between these four experiments and the previous ones are:

- In experiments ST-6, ST-7, and ST-8 toluene and Triton DF-12 were added at the same time to water.
- In experiments ST-12, ST-13, ST-17, and ST-18 toluene was added first to water, mixed, purged with nitrogen, then Triton DF-12 was added, mixed, pumped to the reactor, and purged with nitrogen again.

Experiments ST-9, ST-10, ST-11, and ST-19 -- These experiments determined the stripping rate of toluene in the presence of Marasperse N22. Startup and sampling procedures were the same as for experiments ST-12, ST-13, ST-17, and ST-18 except that Marasperse was used instead of Triton DF-12.

Experiments ST-14 and ST-15 -- These experiments determined the stripping rate of toluene with undissolved 1-octanol (1000 ppm). An oil phase was purposefully desired. The same procedure was applied in these experiments as in experiments ST-12, ST-13, ST-17, and ST-18 with the exception that Triton DF-12 was not used. Since it was desired to test stripping in the presence of an oil phase, 34 mL of 1-octanol was injected to the bottom of the column through the inlet feed valve before the toluene solution was pumped to the reactor.

Experiment ST-16 -- This experiment determined the stripping rate of toluene in the presence of salt (5000 ppm). The same procedure was applied in this experiment as in experiments ST-12, ST-13, ST-17, and ST-18 except that salt solution (140 grams of NaCl in 2 L of water) was used instead of Triton DF-12.

Experiment ST-43 -- This experiment determined the stripping rate of toluene in foul condensate and D FOAM ST. Twelve mL of toluene was added to approximately 26 L of foul condensate in the mixing carboy, and stirred with the power mixer at high speed for 5 min; 15.69 g of D-FoamST was dissolved in 125 mL of foul condensate, added to the toluene solution, and then mixed again for 35 min. The mixture was then pumped to the reactor and deoxygenated by purging with nitrogen.

For reproducible results, the test compound should first be well mixed in water before any secondary contaminant such as surfactant, oil, etc. is added. The solution should then be mixed again, pumped to the reactor, and purged with nitrogen before starting the experiment.

1,4-Dichlorobenzene (DCB) Experiments --

Experiment ST-20 -- This experiment determined the stripping rate of DCB in pure water. Because of 1,4-dichlorobenzene's low solubility (79 mg/L at 20°C) and the difficulty of dissolving it in water, 2 g of 1,4-dichlorobenzene was first dissolved in 100 mL of methanol. The solution was shaken until it was completely clear. The dichlorobenzene in methanol solution was added to 27 L of pure water in the mixing carboy, and stirred with the power mixer at high speed for 1 hr. The solution was then pumped to the reactor and purged with nitrogen for 15 min and air was allowed to diffuse to the reactor at a preset air flow rate.

The same procedure was applied in these experiments as in toluene experiments with the exception that an air sample port was not required. A train of three bubblers was connected at the outlet air line and 150 mL of isooctane in each bubbler was used to absorb 1,4-dichlorobenzene for off-gas analysis. This sampling procedure was used for all 1,4-dichlorobenzene experiments.

Experiments ST-21 and ST-25 -- These experiments determined the stripping rate of DCB in pure water. Due to the interference of methanol in the liquid phase analysis of dichlorobenzene in experiment ST-20, another approach was designed for the preparation of 1,4-dichlorobenzene solution for the rest of dichlorobenzene experiments. Crystalline 1,4-dichlorobenzene was suspended in pure water and mixed by a small stirrer or a magnetic stirrer in several 4-L glass containers. These reached a concentration of about 50 mg/L (or higher in other experiments) one or two days before the experiment. The solution was then filtered through a 20-in. diameter Whatman folded filter paper and mixed by the power mixer to obtain a homogeneous batch. The filtered solution was observed to be completely clear before it was pumped to the reactor and purged with nitrogen.

Experiments ST-24, ST-26, and ST-27 -- These experiments determined the stripping rate of DCB in the presence of Triton DF-12 (0.01%). The procedure was the same as for experiments ST-21 and ST-25 with 2.7 g of Triton DF-12 added to the 26 L already filtered DCB batch and the solution mixed for 2 min before it was pumped to the reactor.

Experiment ST-46 -- This experiment determined the stripping rate of DCB in the presence of toluene. The procedure was the same as for experiments

ST-21 and ST-25. Twelve mL of toluene was added to the 26-L already filtered DCB batch and the solution was mixed for 20 min before it was pumped to the reactor.

Experiment ST-32 -- This experiment determined the stripping rate of DCB in the presence of salt (5000 mg/L). The procedure was the same as for experiments ST-24, ST-26, and ST-27 but 140 g of NaCl was used instead of Triton DF-12.

Experiments ST-28, ST-29, ST-30, and ST-31 -- These experiments determined the stripping rate of DCB in the presence of Tamol 731 (0.01%) and DCB in foul condensate. The same procedure was applied in these experiments as in experiments ST-24, ST-26, and ST-27 except that 2.7 g of surfactant Tamol 731 was used. Because of the high foaming nature of Tamol 731 and foul condensate, a 2-gal polyethylene trap was added at the condenser inlet line for experiments ST-30 and ST-31. DCB in foul condensate was prepared in the same way as DCB in water.

Phenol Experiments --

Experiments ST-22 and ST-23 -- These experiments determined the stripping rate of phenol in pure water. An amount of phenol was dissolved in about 400 mL of water. Concentrated phenol solution was then added to about 26 L of water in the mixing carboy and stirred with the power mixer at high speed. The solution was observed to be completely clear before it was pumped to the reactor and was deoxygenated by purging with nitrogen. Fourteen grams of crystalline phenol were used in experiments ST-22 and 2.8 grams were used in experiment ST-23 to give a starting concentration of 547 mg/L and 107 mg/L for experiments ST-22 and ST-23, respectively. These concentrations were well below the phenol solubility limit. The reactor was covered with aluminum foil during phenol experiments for UV light protection. Experiment ST-22 lasted approximately 4 days, and experiment ST-23 one week.

The same procedure was applied in these experiments as in the 1,4-dichlorobenzene experiments. A train of three bubblers was connected at the outlet air line and 150 mL of 0.1N NaOH was used in each bubbler to absorb phenol for off-gas analysis. The set of bubblers was changed daily. Liquid samples were taken at an interval of 20 to 24 hours.

Beginning with experiment ST-23, water was added to the reactor as samples were removed to maintain a constant liquid volume. Experiments ST-28, 29, and 31 were the exceptions because of foaming problems.

Methyl Ethyl Ketone (MEK) Experiments --

The following startup and sampling procedures were applied to all MEK experiments. A concentration range of 1000 mg/L of MEK was chosen for all MEK stripping experiments. The high MEK solubility limit, 32,260 mg/L at 20°C, allowed it to be dissolved in water without any difficulty. Thirty-four mL of MEK was added to approximately 26 L of pure water in the mixing carboy and was stirred with the power mixer at high speed for 5 min.

The solution was observed to be completely clear before it was pumped to the reactor and was deoxygenated by purging with nitrogen. When surfactant was needed, it was added to the clear MEK solution and mixed again for 5 min before pumping to the reactor. Liquid samples were taken every 4 to 6 hours and each experiment lasted approximately 24 hours.

Procedure for Experiments Using Nonviable Biomass (Experiments ST-42, ST-44, ST-45, and ST-47) --

The startup and sampling procedures for these experiments are described in the preceding experimental procedure section. Nonviable biomass was prepared by lyophilization of municipal sludge, then dry heat treated for 5 hr. The procedure for the preparation of nonviable dried biomass is described in Section 6. Nonviable dried biomass (47.84 g) was added to about 2.5 L of pure water and stirred with a magnetic stirrer for 30 min. It was then pumped to the reactor before pumping the chemical solution. 25.7 L of chemical-biomass solution were added to yield a final concentration of 1860 mg/L of biomass on a dry basis. The chemical-biomass solution in the reactor was purged with nitrogen for about 10 to 15 min before starting an experiment.

DATA ANALYSIS PROCEDURE

Calculation of Stripping Rate Constant, $K_a{}^{st}a$

The value of the stripping rate constant for each experiment was calculated from the measured values of the concentration of the chemical by substitution into the integrated form of the equation below:

$$\text{Stripping rate, } -r^{st} = -\frac{dC_a{}^{st}}{dt} = K_a{}^{st}a \; C_a \qquad \text{Eqn. 36}$$

$$\text{or} \qquad C_a = C_{ao}e^{-K_a{}^{st}a\,(t)} \qquad \text{Eqn. 37}$$

$$\text{or} \qquad \ln \frac{C_{ao}}{C_a} = K_a{}^{st}a \;\; (t) \qquad \text{Eqn. 38}$$

Where C_{ao} and C_a are the chemical concentrations at times o and t, respectively.

A plot of log (C_{ao}/C_a) vs. time t is linear. The slope of the line is $K_a{}^{st}a/2.3$. Therefore, a stripping rate constant, $K_a{}^{st}a$, may be obtained. Two typical graphs for the results of experiments ST-3 and ST-4 with toluene in pure water at an air flow rate of 2 L/min and 4 L/min are shown in Figs. 8 and 9 respectively. The $K_a{}^{st}a$ values were obtained using a linear regression of the form $y = a + b \ln(x)$ for all experiments.

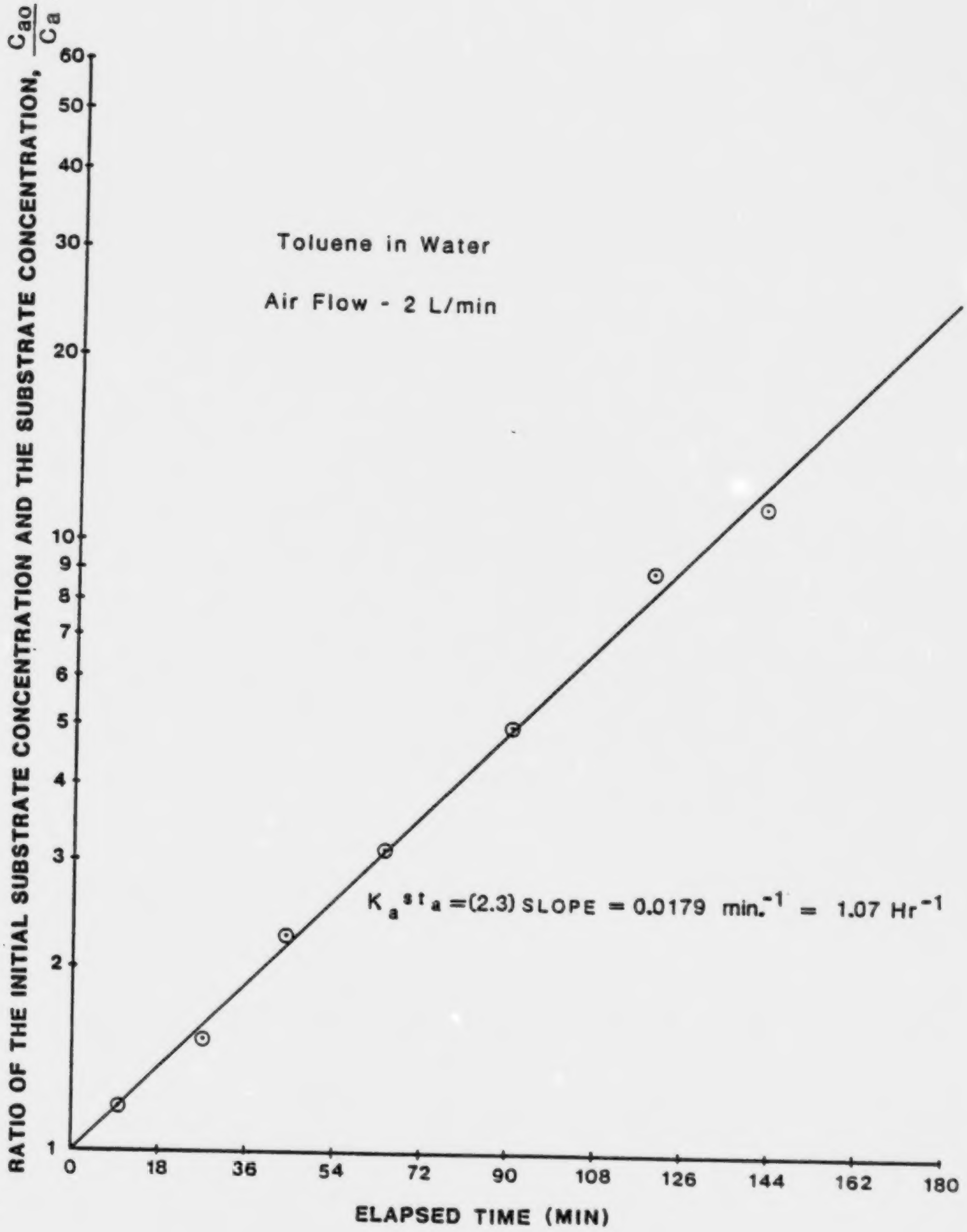


FIG. 8. DETERMINATION OF THE STRIPPING RATE CONSTANT, K_a^{st} a, IN EXPERIMENT ST-3

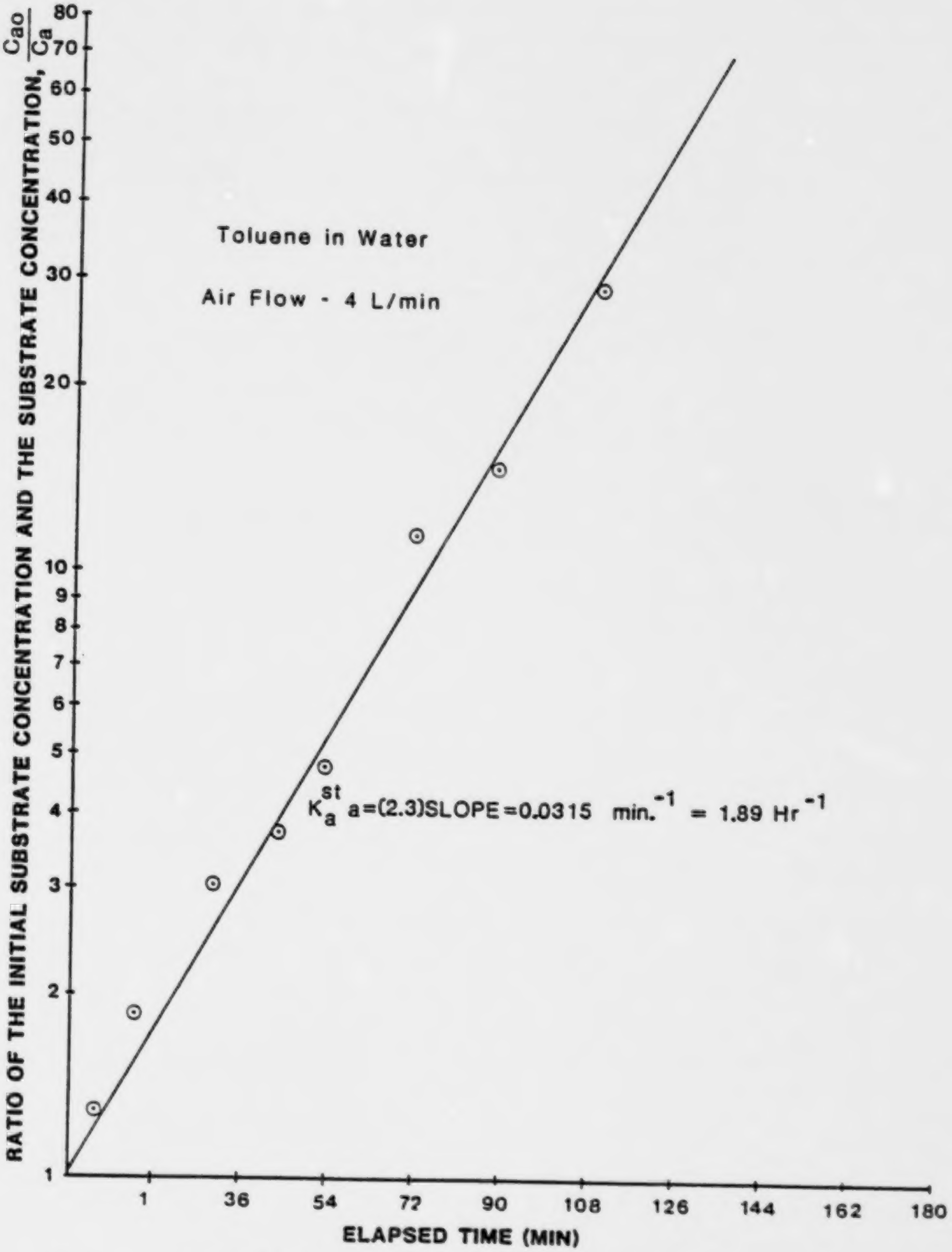


FIG. 9. DETERMINATION OF THE STRIPPING RATE CONSTANT, K_a^{st} a, IN EXPERIMENT ST-4

Calculation of Oxygen Reaeration Rate Constant, K_La

The data for oxygen concentration as a function of time were fitted to the exponential expression for oxygen reaeration.

$$[O_2]_t = [O_2]_s - ([O_2]_s - [O_2]_o)\, e^{-K_La\,(t)} \qquad \text{Eqn. 39}$$

or

$$\ln \frac{([O_2]_s - [O_2]_o)}{([O_2]_s - [O_2]_t)} = K_La \;(t) \qquad \text{Eqn. 40}$$

where:

$[O_2]_s$ = oxygen saturation concentration at the temperature of the solution (mg/L) in tables supplied with the dissolved oxygen probe, and

$[O_2]_o$ and $[O_2]_t$ = oxygen concentrations at times o and t, respectively (mg/L).

Again a plot of log $\{([O_2]_s - [O_2]_o)/([O_2]_s - [O_2]_t)\}$ vs. time t is linear. The slope of the line is $K_La/2.3$. Therefore an oxygen reaeration rate constant K_La may be obtained. Two typical graphs for experiments ST-3 and ST-4 with toluene in water are shown in Figs. 10 and 11, respectively. The K_La values were obtained by using a linear regression for all experiments as noted above.

Material Balance Calculation

Toluene and Methyl Ethyl Ketone Experiments --
Periodic gas samples analyzed for toluene and methyl ethyl ketone were integrated over the runs and compared with initial and end values of the compound in the liquid phase to determine the material balance. A typical calculation for experiment ST-1 is presented in Appendix C.

1,4-Dichlorobenzene and Phenol Experiments --
Phenol and 1,4-dichlorobenzene in the off-gas were collected in a train of three bubblers. Measurements of the volume of the bubblers at the end of each experiment and the concentration of the chemical in each bubbler, and also the concentration and volume of the chemical in the condensed water, were calculated and compared with the initial and end values of the compound in the liquid phase to determine the material balance.

Calculation of Specific Power Input

A power input per volume may be calculated using the following equation:[50]

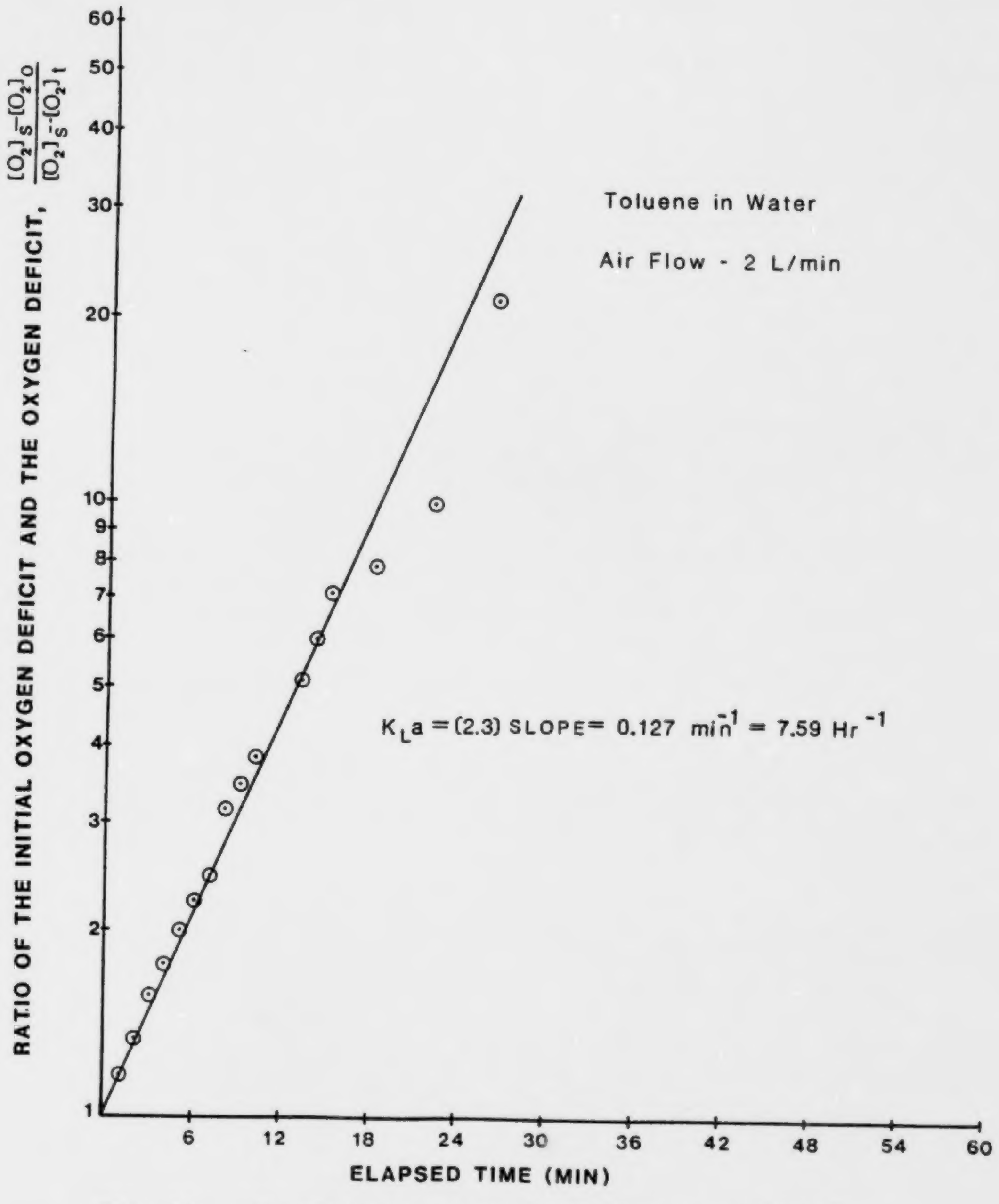


FIG. 10. DETERMINATION OF THE REAERATION RATE CONSTANT, K_La, IN EXPERIMENT ST-3

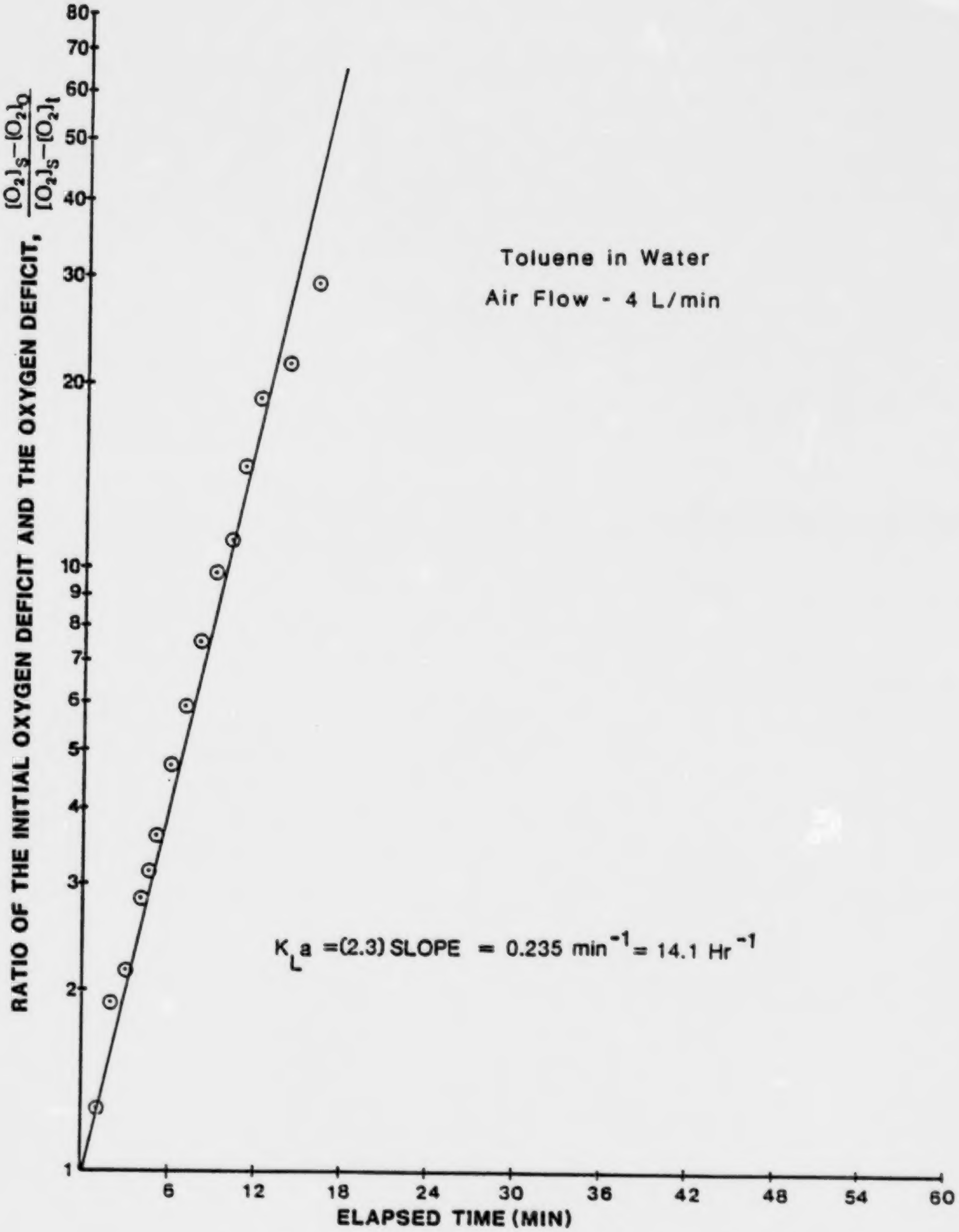


FIG. 11. DETERMINATION OF THE REAERATION RATE CONSTANT, K_La, IN EXPERIMENT ST-4

$$HP = 5.729 \times 10^{-3} \rho_{air} TQ_{air} \left[\left(\frac{15.5 + H_L + H}{14.6} \right)^{0.283} \right]^{-1} \qquad \text{Eqn. 41}$$

where:

HP = air delivered horsepower,

ρ_{air} = air density at the temperature, pressure, and relative humidity for which Q is reported (lb/ft^3),

Q_{air} = air flow (cfm),

T = absolute inlet temperature (°R),

H_L = diffuser headloss corrected to 68°F (psi), and

H = static head (psig).

The specific power of toluene experiments at three flow rates (2, 4, and 8 L/min) can be found in Table 31 and they are a linear function of the air flow rate. Since this relationship is linear and this system is completely mixed, these results should apply to plant designs as long as complete mixing is established. For a completely dissolved substrate, specific power inputs in excess of those needed for complete mixing should only have an impact on stripping where interphase mass transfer is limiting.

TABLE 31. SPECIFIC POWER INPUT FOR TOLUENE EXPERIMENTS

Experiment number	Air flow (L/min)	Specific power (hp/1000 gal)
3	2	0.13
4	4	0.30
11	8	0.57

RESULTS AND DISCUSSION

Toluene Experiments

Experiments ST-1, ST-2, and ST-3 were conducted at the same air flow rate, 2 L/min, but had different oxygen reaeration rate constants. The result of experiment ST-1 was considered unreliable due to the incorrect reading of the initial dissolved oxygen concentration. Tap water was used in experiment ST-2 and deionized (DI) water in experiment ST-3; the results of these two experiments were essentially identical. For convenience, tap water was used for all following stripping experiments and was called "pure water" as noted earlier. Detailed results of all experiments may be found in Appendix C.

The material balance for these experiments were calculated by determining the cumulative material stripped through integration of off-gas flow and concentration data and dividing by the material removed from the aqueous phase, as calculated by difference in the initial and final concentrations. This calculation method is very sensitive in highlighting competing mechanisms or experimental difficulties.

The ratio of stripping rate constant of toluene in pure water over the oxygen reaeration rate constant seemed constant within the limits of these experiments. K_a^{st} and K_La were found approximately linear to the air flow-to-liquid volume of the solution. Values of the stripping rate constant, $K_a^{st}a$, and the oxygen reaeration rate constant, K_La, of all toluene experiments are listed in Table 32. The material balance of experiment ST-6 and ST-7 with toluene in surfactant Triton DF-12 was low. This may have been the result of improper technique used in preparing the toluene-Triton DF-12 solution; therefore, these results are not used. A duplicate of experiments ST-6 and ST-7 performed later in experiments ST-13 and ST-12, respectively, gave better results which fit well in our model.

Experiments ST-3 and ST-13 had a high percent material balance, possibly due to the incomplete toluene mixing procedure and the syringe method of the air analysis, from the Marasperse experiment (ST-13) on. The air bag method was introduced to the system to spotcheck the syringe air analysis during the first 5 min for the remainder of toluene experiments.

The material balances for the remainder of the toluene runs were acceptable with the exception of the 1-octanol experiment, ST-14. That high value may be related to the extraction of toluene by octanol from the liquid phase which leads to low aqueous phase measurements.

1,4-Dichlorobenzene Experiments

The material balance was acceptable in all dichlorobenzene experiments except experiment ST-31 with dichlorobenzene in foul condensate. In this experiment, foul condensate caused excessive foaming problems in the reactor. Therefore, a 2-gal polyethylene foam trap was added at the condenser inlet line to reduce the possibility of contaminating the off-gas samples in the bubblers. In experiment ST-31, loss into the foam trap probably was the result of the low value in the material balance. Again, the stripping rate constants of 1,4-dichlorobenzene are a linear function of the air flow-to-liquid volume of the solution. Values of the stripping rate constants and oxygen reaeration rate constants of all dichlorobenzene experiments can be found in Table 33 along with their material balances and surface tensions.

MEK Experiments

The stripping rate constant of methyl ethyl ketone seemed to be affected very little by the addition of other substances to water. Triton DF-12 at 0.01% and 0.001%, D-Foam ST in foul condensate and nonviable biomass are examples of this. This effect appeared small when compared with the effect

TABLE 32. EXPERIMENTAL DATA OBTAINED DURING STRIPPING RATE MEASUREMENTS FOR TOLUENE

Matrix tested	Experiment No.	Air flow rate (L/min)	Material balance[a] (%)	Stripping rate constant ($K_a{}^{st}a$[hr^{-1}])	Reaeration rate constant (K_La[hr^{-1}])	$\frac{K_a{}^{st}a}{K_La}$	Surface tension (dynes/cm)
Toluene in water	ST-1	2	104	1.13	2.76		68.6
	ST-2	2	100	1.16	7.65	0.151	67.4
	ST-3	2	120	0.97	7.13	0.136	64.0
	ST-4	4	105	1.84	14.00	0.132	58.0
	ST-5	8	82	3.98	31.50	0.126	61.3
Toluene + Triton DF-12 (0.01%)	ST-6	2	76	0.72	1.81	0.397	34.9
	ST-13	2	123	0.58	1.69	0.344	35.8
	ST-7	4	63	1.53	4.58	0.335	35.1
	ST-12	4	102	1.46	4.61	0.318	36.2
	ST-18	5	91	1.99	6.59	0.302	37.0
	ST-17	6	101	2.22	8.01	0.277	35.8
	ST-8	8	93	2.67	12.86	0.208	31.7
Toluene + Marasperse (1%)	ST-9	2	100	0.58	0.91	0.641	51.7
	ST-10	4	81	1.67	4.29	0.388	59.3
	ST-11	8	102	3.31	9.30	0.356	56.9
Mar.[b] (0.5%)	ST-19	2	111	0.63	1.53	0.412	61.5
Toluene + 1-octanol (1000 mg/L)	ST-14	2	268	0.61	2.32	0.263	32.7
	ST-15	8	106	2.48	17.28	0.144	32.1
Toluene + salt NaCl (5000 mg/L)	ST-16	2	154	0.87	4.54	0.191	66.8
Toluene + FC[c] + D-foam (602 mg/L)	ST-43	2	[d]	0.71	2.12	0.335	67.3
Toluene + non-viable biomass (1500 mg/L)	ST-44	2	[d]	0.59	2.12	0.278	60.5
Toluene + FC[c] + D-foam (602 mg/L) + Triton DF-12 (0.01%) + non-viable biomass	ST-45	2	118	0.58	1.19	0.488	43.1
Toluene + 1,4-dichlorobenzene	ST-46	2	83	0.89	5.10	0.175	70.3

---

[a] Material balance calculated as the amount recovered in the off-gas divided by the amount lost from the liquid times 100.

[b] Marasperse.

[c] Foul condensate.

[d] Off-gas analysis was not performed, therefore no material balance was obtained.

TABLE 33. EXPERIMENTAL DATA OBTAINED DURING STRIPPING RATE MEASUREMENTS FOR 1,4-DICHLOROBENZENE

Matrix tested	Experiment number	Air flow rate (L/min)	Material balance[a] (%)	Stripping rate constant ($K_a{}^{st}a[hr^{-1}]$)	Reaeration rate constant ($K_La[hr^{-1}]$)	$\frac{K_a{}^{st}a}{K_La}$	Surface tension (dynes/cm)
DCB in water	ST-20	2	100	0.32	4.83	6.6 X 10^{-2}	60.5
	ST-21	4	129	0.91	16.8	5.4 X 10^{-2}	65.6
	ST-25	8	110	1.94	33.9	5.7 X 10^{-2}	57.7
DCB + Triton DF-12 (0.01%)	ST-24	2	108	0.33	2.03	16 X 10^{-2}	37.0
	ST-27	4	100	0.77	5.20	15 X 10^{-2}	36.4
	ST-26	8	108	1.44	15.8	9.1 X 10^{-2}	37.5
DCB + Tamol 731 (0.01%)	ST-28	2	83	0.41	2.42	17 X 10^{-2}	58.1
	ST-29	4	108	1.17	9.39	12 X 10^{-2}	58.7
	ST-30	8	129	2.03	17.8	11 X 10^{-2}	59.7
DCB + salt NaCl (5000 mg/L)	ST-32	2	101	0.53	5.46	9.7 X 10^{-2}	53.0
DCB + foul condensate	ST-31	2	46	0.31	2.90	11 X 10^{-2}	57.7
DCB + Toluene	ST-46	2	83	0.33	5.10	6.5 X 10^{-2}	70.3
DCB + nonviable biomass (1500 mg/L)	ST-47	2	100	0.32	2.22	14 X 10^{-2}	57.1

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[a] Material balance calculated as the amount recovered in the off-gas divided by the amount lost from the liquid times 100.

of air flow-to-liquid volume of the solution and again $K_a{}^{st}a$ seemed to vary linearly with it. Values of the stripping rate constant, $K_a{}^{st}a$, and oxygen reaeration rate constant K_La of all methyl ethyl ketone experiments are listed in Table 34.

Phenol Experiments

Phenol stripping rates were determined by gas analysis, since water evaporation makes liquid phase analysis unreliable. Table 35 indicates that phenol has no significant stripping as predicted. Kincannon, et al.,[19] found 1.9% of phenol stripped in a nonbiological system. The experimental value for phenol percent removal in our system was 0.71% at a 4 L/min air flow rate over a 4-day run period and 0.79% at the air flow rate of 2 L/min for a 7-day run. Air-to-water ratios in Kincannon's study exceed those used here. The material balance for phenol experiments was very low, probably because phenol strips more rapidly than the water evaporation rate. Liquid concentration data without water loss corrections are unreliable.

All stripping experiments were conducted at temperatures between 17.0°C to 23.6°C. Gaudy[51] found that the stripping rate constant values remain essentially constant for a relative increase in temperature intervals of 5 to 10°C; therefore, it can be assumed that temperature changes had little effect on the experimental stripping rate constant results.

Dispersion Test

In the batch system and at an air flow rate of 2 L/min, water on the top of the reactor reached its equilibrium concentration within 5 to 6 min after the salt solution was injected at the bottom of the column and the time was shorter as air flow rate increased. For example, at an air flow of 6 L/min, it took only 1½ to 2 min for the system to reach a constant concentration. Flow-through analysis gave a large dispersion number, D/uL, of 0.182, 0.190, and 0.196, at air flow rates of 1.5 L/min, 4 L/min, and 8 L/min, respectively. These results indicate a well mixed system approaching complete mixing throughout the air flow range studied.

Stripping Predictive Method

The ratio of the stripping rate constant over the oxygen reaeration rate constant $K_a{}^{st}a/K_La$ was constant for the toluene experiments in pure water, as predicted by Smith, et al.[44,45] for high volatility compounds in natural waters. But this constant value varied greatly with the addition of various chemicals. The ratio, $K_a{}^{st}a/K_La$ = constant, does not appear to be a reliable predictor for stripping rates in actual wastewater treatment processes.

The results from Tables 32 through 35 for toluene, 1,4-dichlorobenzene, and methyl ethyl ketone, each at three levels of air flow rates of 2, 4, and 8 L/min and also for phenol in pure water at an air flow rate of 2 L/min and 4 L/min may be correlated to produce a simple power relationship. This

TABLE 34. EXPERIMENTAL DATA OBTAINED DURING STRIPPING RATE MEASUREMENTS FOR METHYL ETHYL KETONE

Matrix tested	Experiment number	Air flow rate (L/min)	Material balance[a] (%)	Stripping rate constant ($K_a^{st}a[hr^{-1}]$)	Reaeration rate constant ($K_La[hr^{-1}]$)	$\frac{K_a^{st}a}{K_La}$	Surface tension (dynes/cm)
MEK in water	ST-33	8	89	0.038	30.2	1.3×10^{-3}	57.5
	ST-34	4	93	0.019	12.8	1.5×10^{-3}	59.5
	ST-35	2	104	0.009	5.26	1.7×10^{-3}	60.6
MEK + Triton DF-12 (0.01%)	ST-36	4	89	0.018	4.74	3.8×10^{-3}	36.1
	ST-37	2	101	0.008	1.69	4.7×10^{-3}	36.4
	ST-38	8	102	0.035	13.1	2.7×10^{-3}	36.2
MEK + Triton DF-12 (0.001%)	ST-39	2	90	0.011	1.78	6.2×10^{-3}	42.9
	ST-40	4	90	0.019	4.96	3.8×10^{-3}	45.5
MEK + FC[b] + D-foam (602 mg/L)	ST-41	2	97	0.009	0.81	11.1×10^{-3}	63.3
MEK + nonviable biomass (1500 mg/L)	ST-42	2	90	0.013	2.40	5.4×10^{-3}	63.5

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[a] Material balance calculated as the amount recovered in the off-gas divided by the amount lost from the liquid times 100.

[b] Foul condensate.

TABLE 35. EXPERIMENTAL DATA OBTAINED DURING STRIPPING RATE MEASUREMENTS FOR PHENOL

Matrix tested	Experiment No.	Air Flow rate (L/min)	Material balance[a] (%)	Stripping rate constant ($K_a^{st}a$ [hr^{-1}])	Reaeration rate constant (K_La [hr^{-1}])	Surface tension (dynes/cm)
Phenol in water	ST-22	4.0	16.8	8.10×10^{-5}	13.7	53.6
	ST-23	2.0	7.0	5.12×10^{-5}	7.08	64.0

[a] Material balance calculated as the amount recovered in the off-gas divided by the amount lost from the liquid times 100.

relationship incorporates Henry's law constant and the air flow-to-liquid volume ratio to directly yield the stripping rate constant for pure water systems.

$$K_a^{st}a \cdot \frac{V}{Q_{air}} = b(H_c)^m \qquad \text{Eqn. 42}$$

where:

$K_a^{st}a$ = stripping rate constant (hr^{-1}),

H_c = Henry's law constant $\left(\frac{\text{torr L}}{\text{g-mole}}\right)$,

V = volume of the liquid in the reactor (L),

Q_{air} = air flow rate (L/min),

b and m = power function constants, and

$K_a^{st}a \cdot V/Q_{air}$ = stripping rate parameter.

Table 36 presents the findings for these equations. For pure water systems, $b = 3.71 \times 10^{-3}$ and $m = 1.045$ with 11 data points yields a correlation coefficient, r^2, of 0.991.

TABLE 36. EMPIRICAL RELATIONSHIP BETWEEN STRIPPING KINETIC RATE CONSTANT AND HENRY'S LAW CONSTANT FOR DIFFUSED AERATION SYSTEMS

Data included	Equation 42 constants		Number of data points	Regression coefficient r^2
	b	m		
Toluene, 1,4-dichlorobenzene, and MEK in pure water at air flow rate 2,4,8 L/min and phenol in pure water at air flow rate 2,4 L/min	3.71×10^{-3}	1.045	11	0.991
Toluene, DCB, and MEK in Triton DF-12 (0.01%) at air flow rate 2,4,5,6, and 8 L/min	9.30×10^{-3}	0.872	11	0.997

Use of the dimensionless Henry's law constant yields the simple dimensionless relationship:

$$K_a^{st}a \cdot \frac{V}{Q_{air}} = H_i \qquad \text{Eqn. 43}$$

Reconciliation of this equation with two-film theory suggests that mass transfer in the system is very rapid, reaching equilibrium quickly. This result is related to the turbulence in an "engineered" system and leads to the conclusion that stripping in the dispersed phase system (gas or liquid) may be fundamentally different from volatilization, where mass transfer processes are limiting.

Roberts, et al.,[52] in work done roughly simultaneously with this work, derived an equation for bubble columns with fine and coarse bubble diffusers. In compatible symbols with this report:

$$-\ln\left(\frac{Ca}{Cao}\right) = \frac{Q_{air}}{V} \cdot H_i\ (1 - e^{\Phi z_s})\ (t-t_o) \qquad \text{Eqn. 44}$$

where:

C_{ao} and C_a = the initial and final liquid concentration for the substrate, respectively,

Q_{air} = the gas flow rate (m^3/min),

V = the liquid volume (m^3),

H_i = the mass concentration ratio unitless Henry's law constant,

Z_s = diffuser depth (m),

t_o and t = the initial time and elapsed time (min), and

Φ = a saturation parameter equal to $K_a^{st}a \cdot V/H_i \cdot Q_{air}$.

The following equation can be derived from Eqn. 44, considering the batch reactor design equation (Eqn. 12):

$$K_a^{st}a \cdot \frac{V}{Q_{air}} = H_i\ (1 - e^{\Phi z_s}) \qquad \text{Eqn. 45}$$

When $\Phi z_s > 5$, the gas phase is saturated and Eqn. 45 reduces to Eqn. 43.

In work in a continuously mixed batch reactor, Baillod and his colleagues[53] demonstrated that the first-order kinetic rate constant for substrate removal by stripping during an ozonation reaction is consistent with Eqn. 43. This work[53] couples the kinetics of a chemical oxidation (ozonation) with the stripping mechanism.

With the weight of these studies and our work it seems clear that stripping from a diffused air biological reactor will closely approximate an equilibrium process with one equilibrium stage.

Table 36 also presents the power function constants for three compounds in surfactant Triton DF-12 solution with 11 data points and a high regression coefficient value.

This model for our stripping PFM was tested in the lab-scale activated sludge study using toluene in a continuous system with a liquid volume of 11 L and a mean air flow rate of 2 L/min in a similar bubble size distribution. The predictor developed in this study for pure water, $K_a^{st}a \cdot V/Q_{air} = 3.71 \times 10^{-3}(H_c)^{1.045}$ gave the stripping rate constant predicted for toluene in the lab-scale activated sludge system of 2.7 hr^{-1}. This predicted value provides good agreement as compared to an experimental mean of 2.4 hr^{-1} for both biological and nonbiological systems. The lab-scale systems operated during several upset conditions, but the stripping rate constant remained relatively constant. This relationship was tested to the detection limit of 50 ppb in water and was found to be accurate. Comparisons found in Section 10 also verify the stripping model, even though daily average concentration data used led to greater error.

This model was also tested to predict the stripping rate constant in previous studies. Kincannon, et al.,[19] conducted a bench-scale continuous flow study on a biological and a nonbiological system. In his nonbiological system for the stripping study, distilled water was used in a 6.13-L stainless steel reactor. Liquid flow rate to the reactor was varied from 5.8 mL/min to 6.4 mL/min. The air flow rate to the reactor varied from 2.0 L/min to 3.0 L/min. The values of the stripping rate constant $K_a^{st}a$ calculated from Kincannon's reported data for five compounds are presented in Table 37 along with the values of $K_a^{st}a$ predicted from the model of this study for pure water. These values seem to be in good agreement.

Roberts[54] conducted an experimental study of the volatile organic contaminants from aqueous solution during surface aeration. A cylindrical glass vessel open to the atmosphere with blade impellers for the stirrer device was used. The volume of water used was 7.3 L at a constant temperature of 20±1°C. Roberts stated that the value of the stripping rate constant $K_a^{st}a$ increased nearly linearly with the specific power input (watt/m^3) in the range of 10 watt/m^3 and 200 watt/m^3. The values $K_a^{st}a$ calculated from Roberts' data were obtained by using the specific power input, 59.5 watt/m^3, calculated for the toluene experiment in this study, at an air flow rate of 4 L/min. With this specific power input value applied to Roberts' system of 7.3 L liquid volume for a power of 0.435 watt, the values of the stripping rate constant of the compounds can be found. These values are shown in Table 37 and, except for the very high volatility compound tetrachloroethylene, they seem to agree reasonably well with the predicted values.

Weber[35] provides another opportunity for comparison. Weber used a 10-liter batch reactor at a temperature of 20°C and an air flow rate ranging

TABLE 37. COMPARISON OF REPORTED VALUES OF THE STRIPPING RATE CONSTANT, $K_a^{st}a$, WITH OTHER COMPLETE-MIXED DATA

Compound	Henry's law constant $\left(\frac{\text{torr L}}{\text{g-mole}}\right)$	Values of $K_a^{st}a(hr^{-1})$ calculated from reported data			$K_a^{st}a(hr^{-1})$ predicted from this study
		Kincannon[19]	Roberts[53]	Weber[35]	
Methylene chloride	2424[a]	9.73			6.14
Chloroform	2388[b]		2.92		1.95
1,2-Dichloropropane	2143[a]	4.94			4.50
Trichloroethylene	7174[b]		3.32		6.16
Benzene	4218[a]			5.46	9.11
Benzene	4218[a]	8.58			10.0
1,1,1-Trichloroethane	3033[b]		3.27		2.51
Chlorobenzene	2359[c]			3.72	4.97
Tetrachloroethylene	15480[b]		3.27		13.8
o-Xylene	3034[b]			4.38	6.46
Toluene	2769[c]			5.58	5.87
Ethylbenzene	4890[b]			6.18	10.6
Phenol	0.25[c]	0.0011			0.0003
1,2-Dichlorobenzene	1149[c]			2.46	2.34

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[a] From ref. 34.

[b] Calculated by using Dilling's equation[47] with solubility and vapor pressure values from ref. 41.

[c] Calculated by using Dilling's equation[47] with solubility and vapor pressure values from ISHOW data base.[48]

from 3 to 5 L/min. The values of the stripping rate constant $K_a^{st}a$ for six compounds in deionized water at air flow rate of 4 L/min reported by Weber can be found in Table 37 along with the values predicted from the model of this study. Again, these values seem to be in good agreement.

Petrasek[34] performed a pilot-plant study to evaluate the removal and partitioning of volatile organic priority pollutants in publicly owned treatment works (POTWs). The pilot plant consisted of a primary clarifier, a 58.3 m^3 plug flow aeration basin, followed by a secondary clarifier. Conventional coarse bubble diffusers were used with the nominal air flow of 57 L/sec. Table 38 lists the values of the stripping rate constant $K_a^{st}a$ calculated from Petrasek's reported data. These calculated values are lower and range from 0.6% for tetrachloroethylene to 94% for dibromochloromethane, as shown in Table 38 for comparison with the predicted values from this study.

These differences are not surprising. Aqueous phase data were measured only at the aeration basin influent and effluent, even though the plant was operated as plug flow. An average of influent and effluent was used in our calculations and was probably much higher than the true mean C_a for the plant. For application of this predictor to plug flows or batch processes, a true mean liquid concentration would have to be determined by sampling the liquid phase at various points within the process, or in the case of batch design, sampling of the liquid over the batch interval. Influent and effluent data are inadequate for prediction of stripping in batch and plug flow reactors. Further discussion of this problem is given in Section 1.

Effect of Contaminants on the Stripping Rate Parameter

To illustrate the relative effects of various contaminants on the stripping rate, the mean values of the stripping rate parameter, $K_a^{st}a \cdot V/Q_{air}$, for all experiments were calculated. In Figs. 12 through 15, the percentages of the mean of $K_a^{st}a \cdot V/Q_{air}$ of toluene, 1,4-dichlorobenzene, and methyl ethyl ketone in various test contaminant systems to pure water are plotted against the mean surface tensions of the related solutions. Standard deviations as a measure of error are also shown.

Figure 12 shows the effects of salts and oils; Fig. 13 shows the effect of surfactants; Fig. 14 shows the effect of biomass; and Fig. 15 shows the effect of a waste stream such as foul condensate on the pure water stripping rate.

Most contaminants caused a reduction in the stripping rate. Systems showing increases are 1,4-dichlorobenzene (DCB) plus salt and methyl ethyl ketone (MEK) with Triton DF-12 and with biomass. The high value in the DCB plus salt experiment may be caused by a decrease in water solubility related to the "salting out" effect. A lower water solubility would tend to increase the Henry's law constant and the stripping rate. The high MEK plus Triton DF-12 data may be artificial. The error bands for the stripping rate parameter ratio overlap those of pure water MEK experiments.

TABLE 38. COMPARISON OF REPORTED VALUES OF THE STRIPPING RATE CONSTANT, $K_a{}^{st}a$, WITH PLUG-FLOW DATA

Compound	Henry's law constant $\left(\frac{\text{torr L}}{\text{g-mole}}\right)$	Values of $K_a{}^{st}a\,(\text{hr}^{-1})$ calculated from Petrasek's reported data	Values of $K_a{}^{st}a\,(\text{hr}^{-1})$ predicted from this study	Percent Agreement $\frac{K_a{}^{st}a \text{ calc.}}{K_a{}^{st}a \text{ pred.}} \times 100$
1,1-Dichloroethene	11400[a]	0.088	3.766	2.3
Chloroform	2388[b]	0.040	0.736	5.4
Carbon tetrachloride	22952[a]	0.071	7.822	0.9
1,2-Dichloropropane	2143[a]	0.038	0.657	5.8
Trichloroethylene	7174[b]	0.062	2.321	2.7
1,1,2-Trichloroethane	562[a]	0.026	0.162	16.1
Dibromochloromethane	593[a]	0.162	0.172	94.2
Benzene	4218[a]	0.026	1.333	2.0
1,1,1-Trichloroethane	3033[b]	0.075	0.944	7.9
Bromodichloromethane	1611[a]	0.127	0.488	26.0
Chlorobenzene	2359[c]	0.006	0.726	0.8
Tetrachloroethylene	15480[b]	0.031	5.184	0.6
1,1,2,2-Tetrachloroethane	289[a]	0.031	0.081	38.3
Toluene	2769[c]	0.024	0.859	2.8
Ethylbenzene	4890[b]	0.025	1.555	1.6

[a] From ref. 34.

[b] Calculated by using Dilling's equation[47] with solubility and vapor pressure values from ref. 41.

[c] Calculated by using Dilling's equation[47] with solubility and vapor pressure values from ISHOW data base.[48]

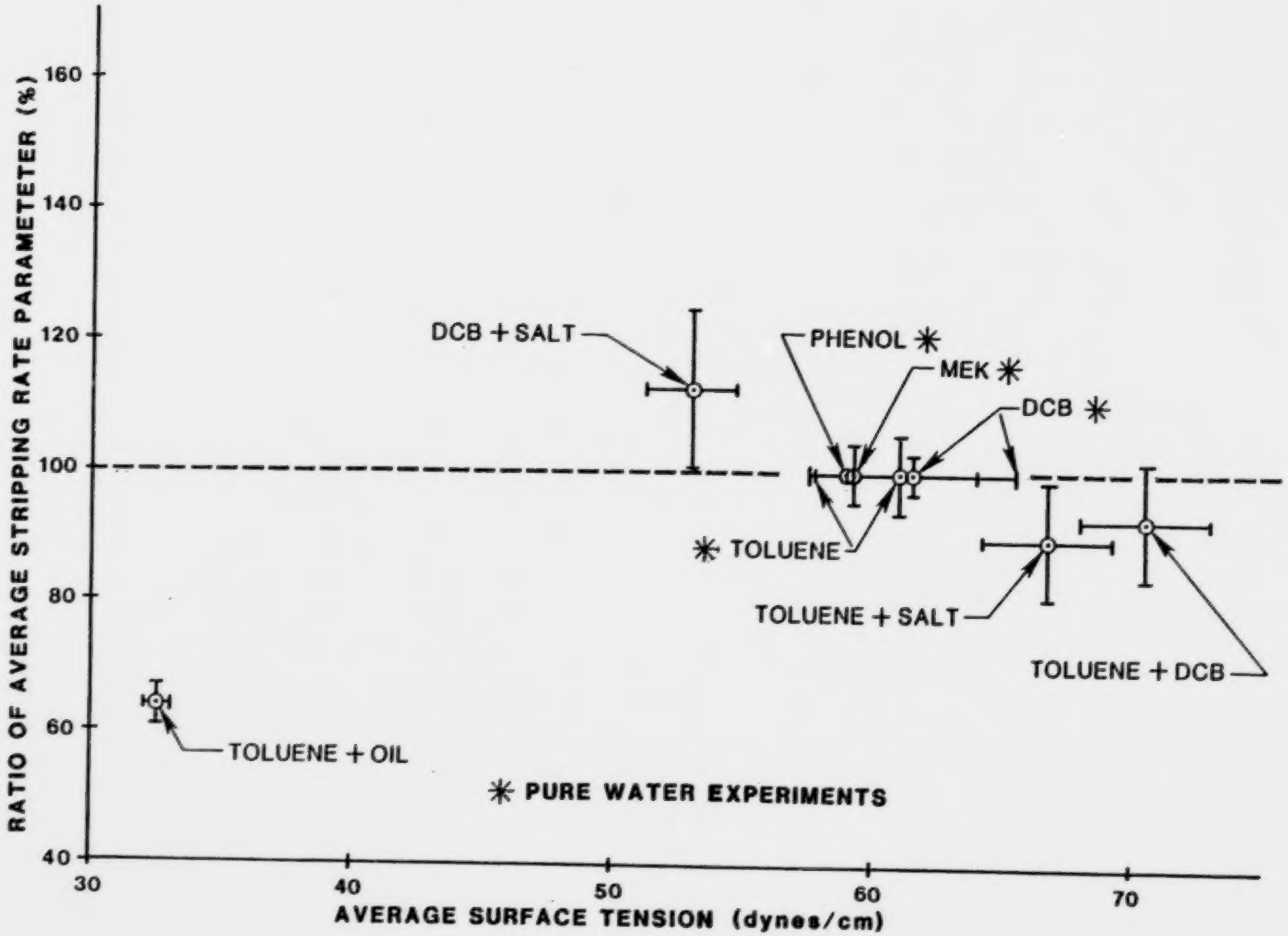

FIG. 12. THE EFFECT OF CONTAMINANTS ON THE SUBSTRATE STRIPPING RATE (PURE WATER, OIL, AND SALT EXPERIMENTS)

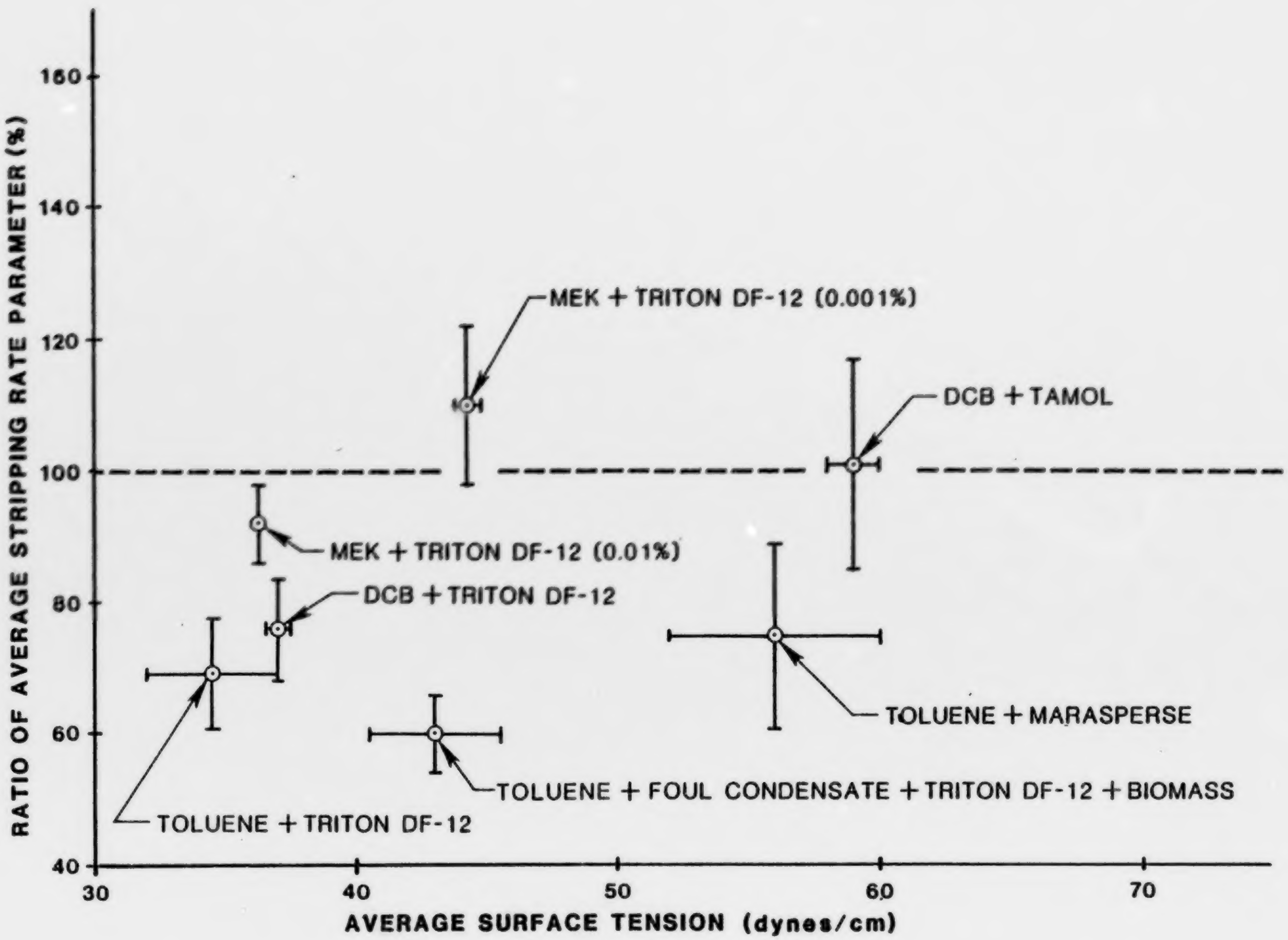

FIG. 13. THE EFFECT OF CONTAMINANTS ON THE SUBSTRATE STRIPPING RATE (TRITON DF-12, MARASPERSE, AND TAMOL SURFACTANT EXPERIMENTS)

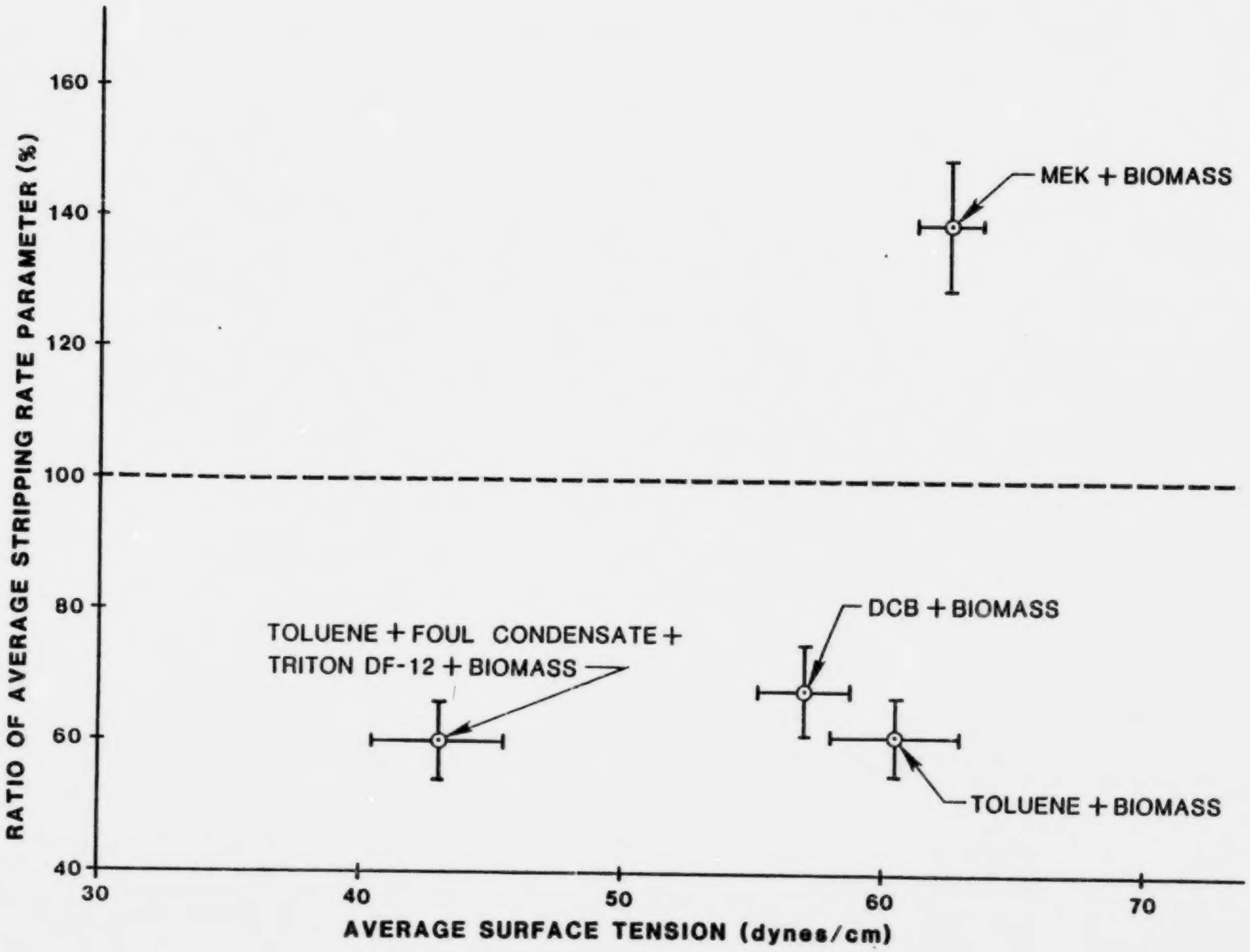

FIG. 14. THE EFFECT OF CONTAMINANTS ON THE SUBSTRATE STRIPPING RATE (BIOMASS EXPERIMENTS)

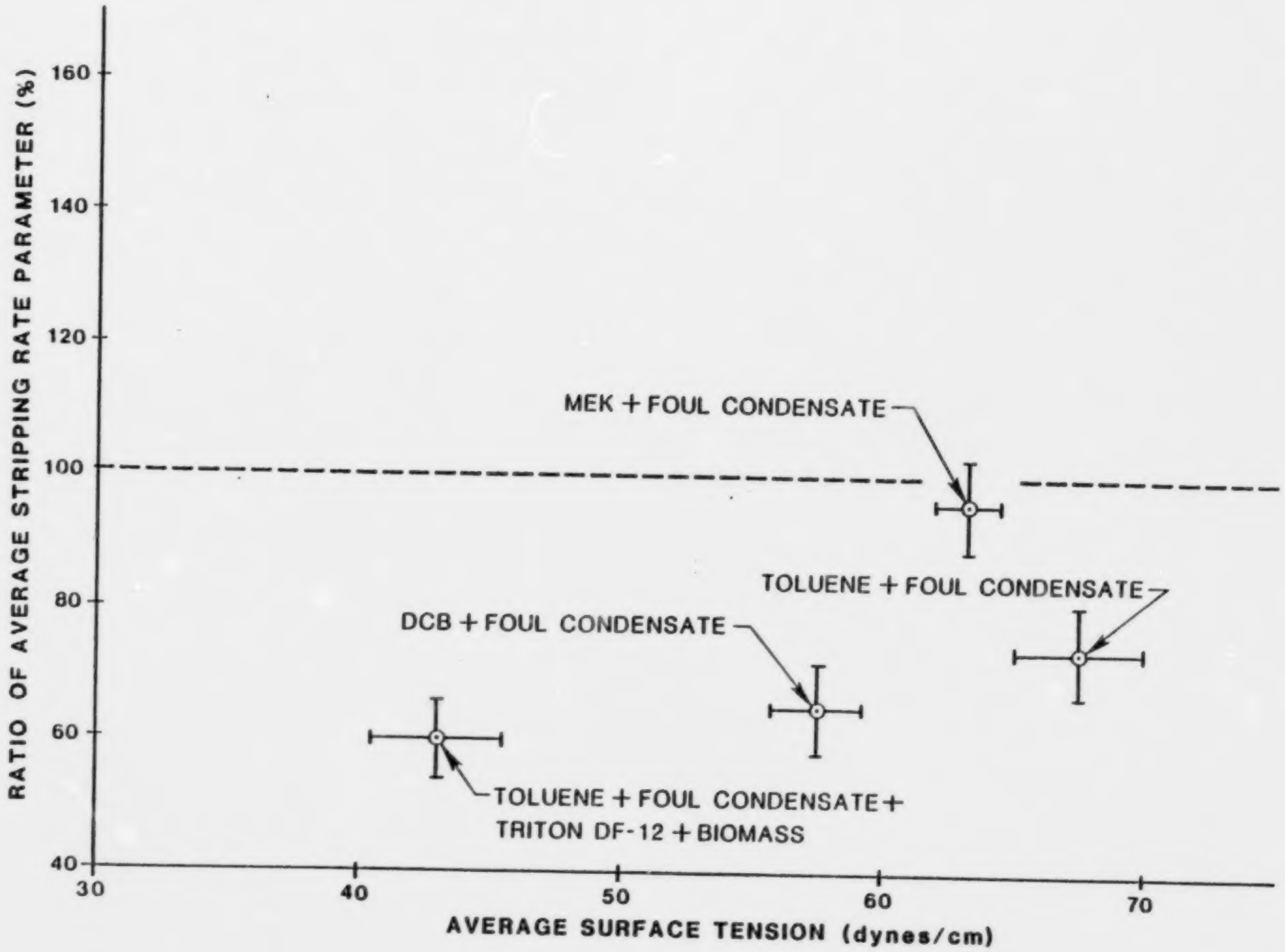

FIG. 15. THE EFFECT OF CONTAMINANTS ON THE SUBSTRATE STRIPPING RATE (FOUL CONDENSATE EXPERIMENTS)

We can give no explanation for the high MEK plus biomass data. The biomass was tested and verified as nonviable using a respirometric technique. Therefore, no bio-oxidation interference is suspected.

In any case, the stripping rate related to other contaminants in the wastewater may vary ±50% when compared with the pure water. The effects also seem to be stronger for the higher volatility compounds.

Surface tension data were collected and included in the data analysis because the variations in stripping rates related to contaminants were expected to be related to the aqueous phase surface tension. A careful analysis of the stripping rate data, the surface tension data, and related error factors yields no clear empirical relationship. However, further tests specifically designed to explore these relationships may be useful since this work was limited in the level of effort available for this problem. A more accurate predictor of water contaminant effects may be needed in cases where the stripping rate and bio-oxidation rates are of the same magnitude.

QA/QC Results

The stripping rate constants, $K_a^{st}a$, and the oxygen reaeration rate constants, K_La, for all toluene experiments are listed in Table C-3 in Appendix C, along with the number of data points analyzed. The values of the coefficient of determination or regression coefficient r^2 generally exceeded 0.99 for K_La in all stripping experiments. In the toluene experiments, r^2 for all $K_a^{st}a$ exceeded 0.99 except in experiment ST-14 with toluene in oil (1-octanol) where $r^2 = 0.95$. The number of data points used for analyzing K_La was never less than 10; the highest was 23. Less data points were used for analyzing the stripping rate constant, $K_a^{st}a$, especially for low volatility compounds such as phenol.

The ratio value of $K_a^{st}a/K_La$ seemed constant within the toluene experiments in pure water where the mean value was 0.131 and the percent variation from the mean was 3.8%. But this ratio departs from its constant value with the addition of other chemicals such as surfactants. This departure can be seen from the 18% variation for the toluene-Triton DF-12 experiment and the 34% variation for the toluene-Marasperse experiment.

Over 35 oxygen transfer experiments were performed at three different flow rates (2, 4, and 8 L/min) to evaluate the oxygen reaeration rates of the stripping experiments. Each oxygen transfer test was performed before the stripping experiments were done during that day, using deoxygenated tap water with no chemicals added. Data for the air transfer testing related to the stripping experiments at air flow rates of 2, 4, and 8 L/min can be found in Tables C-4, C-5, and C-6 in Appendix C. The values of K_La for 23 oxygen transfers at an air flow rate of 2 L/min ranged from 3.65 hr^{-1} to 7.74 hr^{-1}, with the mean being 4.83 hr^{-1} and the percent variation 18%. For eight oxygen transfers performed at air flow rate 4 L/min, the mean value of K_La was 13.0 hr^{-1} and the percent variation was 12%. For five oxygen transfers at flow rates of 8 L/min, the mean value of K_La was 28.6 hr^{-1} and the percent variation was 20%. These variations resulted from different effects during the experiments. These effects may have been due to the quality of tap water each day, to changes in pressure and temperature, and especially to the fact that the dissolved oxygen probe could not be easily moved from the reactor for calibration. Jackson[55] predicted that the oxygen reaeration rate constant (K_La) increases with temperature at a rate about 2%/°C in the range of 0° to 30°C, the average temperature for air transfer in this system was 19°C with a deviation of 2°C. Therefore, it can be concluded that the maximum percentage of experimental variation from day to day for this stripping system can reach a value of 20 to 25%, and lower if the effects mentioned above can be avoided. It is believed that a majority of this system error relates to variations in the dissolved oxygen measurement but the equipment design made it impractical to calibrate the D.O. probe daily. The true error related to only daily variations (analysis excluded) may be substantially less than 20%.

One hundred percent of the QA related data, which includes instrumental precision, method precision, accuracy, and audit sample results, met the project QA objectives. The QA objectives were intended to provide data at EPA quality level 3. This level of quality assurance is appropriate for projects of this type which are intended to evaluate basic options or to perform preliminary assessments of unexplored areas (refer to "Interim Guidelines and Specifications for Preparing Quality Assurance Project Plans," QAMS-005/80, U.S.E.P.A, PB83-170514, 1983).

The project QA objectives along with the detailed QA results for the entire program are included as part of this report in Appendix D.

SECTION 7

PREPARATION OF NONVIABLE BIOMASS

SUMMARY

Biologically inactive (nonviable) biomass was required for conducting experiments on the biological sorption of organics. Five techniques were tested: gamma irradiation, formaldehyde treatment, lyophilization, lyophilization followed by dry heating, and lyophilization followed by exposure to UV light. Biomass from the acclimation reactors was used in all experiments. Of the methods tested, the most desirable was lyophilization of activated sludge followed by dry heating at 105°C for 3 hours. This method produces a stable, dry product that retains the macroscopic physical flocculation and settling properties of the viable biomass and appears to have a long shelf life.

BACKGROUND

Previous studies involving the sorption of organics onto biological solids failed to isolate the various mechanisms that contribute to the disappearance of organics from the aqueous phase. One such mechanism is the biological transformation or oxidation of the organic. Control of the enzymatic processes of the cell is required in order to measure substrate uptake in the absence of biotransformation or bio-oxidation. A method to prepare samples of biologically inactive biomass, having as many similar properties to live biomass as possible, was sought. This inactive biomass was used in sorption studies as described in Section 8.

The literature was surveyed to determine effects of storage atmosphere and preservation techniques or mediums on the viability of microorganisms. Heckley and Blank[56] discussed the viability of freeze-dried microbes prepared from equal volumes of a 6% lactose solution and a broth culture of microbes. Cox and Heckley[57] related the effect of oxygen on freeze-thawed bacteria. The viability of the cells and free radical formation in the cells as a result of the presence of oxygen during the freeze-thaw cycle was studied.

The most comprehensive paper was a chapter from a book on preservation of microorganisms, edited by R. J. Heckley.[58] This chapter reviewed various methods of preservation with reference to the ability of microbes to reproduce, their functional properties, and their maintenance of genetic integrity after preservation. Freezing preservation techniques are the most commonly used, but many factors affect the ability of the cells to survive. These factors include the age of the culture, rate of freezing or thawing, storage temperature, and presence of protective agents.

Dehydration is also a common method for preserving microbes, but lyophilization is the most widely accepted procedure. Even when carefully done, cell kills in excess of 90% are common. The same factors that affect survivability in freezing are present here, with the addition of residual moisture and the effect of oxygen in the storage atmosphere.

MATERIALS AND METHODS

In each of the methods investigated, biological solids were acquired from a set of two identically operated and carefully controlled initial acclimation reactors. The operation and nature of the reactors is detailed in Section 5. Except where noted, each technique used a Gilson Single Valve Differential Respirometer to detect the presence or absence of biological activity.

Solids used in each of the experiments were harvested from each of two bio-reactors. Mixed liquor was transferred from the reactors to a glass 50-L battery jar. After allowing the solids to settle to the bottom of the jar, the supernatant was siphoned to the drain. Solids were then centrifuged in a JEC Centra 7R centrifuge at 1000 rpm. Solids were separated from the liquid, and the solids were retained for use in the experiments. Additional processing of the solids, when needed, is detailed in the experimental procedures.

Respirometric Analysis

The differential respirometer provides a way to detect biological activity by measuring the oxygen demand of a sample. Samples are prepared for measurement on the respirometer by adding biological solids to deionized water so that a 1000 mg/L dry weight of solid concentration is achieved. The sample is then placed in a reaction flask in the respirometer, and the volume of oxygen uptake is measured over time.

Gamma Irradiation Method

Centrifuged solids were transferred to 8-oz clear glass, wide mouth jars. Teflon-lined caps were put on and the closed jar was sealed at the cap with paraffin. The samples were taken to the Oak Ridge Associated Universities, Office of Safety and Health, Department of Health Physics, where a Cobalt 60 variable dose rate irradiation device was available. Forty-five samples were arranged around two sources in such a way that an even distribution of radiation could be achieved. The samples were irradiated for 48 hr and received a total of about 2 Mrads of radiation.

Formaldehyde Exposure Method

Centrifuged solids were transferred to two 4-L Erlenmeyer flasks and filled to the 2 L mark. One liter of 24% formaldehyde solution and one liter of deionized water was added to each flask. The resulting concentration of formaldehyde in each flask was approximately 7% (70,000 mg/L). A stir bar/magnetic stirrer was used to agitate the system. The system was stirred for 4 hours. The mixtures from each flask were transferred to a 50-L glass battery jar. The solids were washed with deionized water four times. After each washing, solids were allowed to settle and the supernatant was removed by siphon. Formaldehyde concentration in the liquid after the final wash was calculated to be approximately 76 mg/L.

Solids were further concentrated by centrifugation at 2000 rpm. A fraction of the treated solids was added to a volume of deionized water so that a 1000 mg/L dry solid weight solution was made. Two 3-mL aliquots of this solids mixture were added to respirometry flasks and analyzed for biological activity.

Lyophilization Methods

Three of the methods investigated used lyophilized (freeze-dried) biomass. Lyophilized material prepared in the same way was used in each of these methods.

To prepare the lyophilized material, 1 L aliquots of centrifuged solids were transferred to 2-L freeze-drying flasks. Freeze drying flasks were placed in a freezer on a battery-operated shell freezer. After the contents were frozen, the flasks were placed on a Labconco Freeze Dryer Model 18 and allowed to dry. Samples usually required 4 to 5 days to completely dry. The dried material was sifted through a No. 8 mesh screen.

Three methods were investigated using the dry product. One method checked the viability of the dry, sifted material with no further processing. Another method further processed the dry, sifted material by heating it in open trays at 105°C for various lengths of time. The third method exposed the dry sifted material in foil-lined, open trays to a UV light source delivering 620 $\mu W/cm^2$ for varying lengths of time.

The solids from each of these three methods were tested for bio-activity in a manner similar to that described above.

RESULTS AND DISCUSSION

Table 39 presents the activity of the biomass for each of the methods tested as measured by respirometry.

Gamma Irradiation Method

The 2 Mrad radiation dosage applied to the biomass sample rendered the biomass inactive. However, the dosage was so extreme that total disruption

TABLE 39. RESPIROMETER RESULTS, VARIOUS TREATMENT METHODS

Treatment method	Time (min)	Cumulative Oxygen uptake (µL)	
Gamma irradiation	a	a	
Formaldehyde treated	0 to 1256	0	
Lyophilized	0	0[b]	0[b]
	45	2	1
	282	18	15
	520	35	30
	1275	170	149
	1436	205	178
	1594	217	194
	1760	251	216
Lyophilized, heated at 105°C for 3 hours	0 to 1256	0	
Lyophilized, heated at 105°C for 5 hours	0 to 1256	0	
Lyophilized, heated at 105°C for 24 hours	0 to 1379	0	
Lyophilized, UV light treated for 5 minutes	0	0	
	45	3	
	282	17	
	520	40	
	1275	192	
	1436	217	
	1594	237	
	1760	260	
Lyophilized, UV light treated for 30 minutes	0	0	
	46	0	
	284	17	
	520	35	
	1275	204	
	1438	239	
	1596	266	
	1761	291	
Lyoohilized, UV light treated for 2 hours	0 to 1390	0	
Lyophilized, UV light treated for 27 hours	0 to 1256	0	

[a] No respirometry measurements.

[b] Duplicate samples measured.

of the solids occurred. Cell damage was so great that even after centrifugation, few solids could be settled from the mixture. Inability to develop an appropriate dose-response relationship because of the logistical difficulties of using the radioactive source ruled out use of this procedure as an expedient means of producing nonviable biomass.

Formaldehyde Treatment Method

The formaldehyde treated biomass showed no signs of biological activity when tested on the respirometer, indicating that this method was successful. However, the processing steps necessary to prepare the solids involved handling large volumes of liquids and solids containing formaldehyde and were too time-consuming. A simpler, faster method was sought.

Lyophilization Methods

Three methods of testing lyophilized material were investigated. Lyophilized biomass used as is (without further processing) showed biological activity upon rehydration. Lyophilized biomass processed with UV light proved to be a suitable method of killing the sludge if exposure times in excess of 2 hr were used and thin, stirred layers were maintained. Dry heating of lyophilized biomass also proved to be an acceptable technique if the solids were heated for at least 3 hr at 105°C. No weight changes were noted in the dry heating step.

Dry heating of lyophilized material was determined to be the simplest method for inactivating biological solids. After rehydration, the lyophilized material resembled the live biomass in macroscopic flocculation and settling properties and had a long shelf life. Therefore, large quantities of the material could be prepared at one time and stored for future use.

SECTION 8

DEVELOPMENT OF A BIOMASS SORPTION PREDICTIVE FATE METHOD

SUMMARY

Sorption experiments were conducted to determine sorption kinetic rate constants and equilibrium relationships between aqueous phase concentration and loadings onto biological solids for three test compounds. Test compounds were chosen to span a wide range in potential for sorption as indicated by the octanol-water partition coefficient K_{ow} (unitless wt/vol concentration ratio). Compounds which were studied include phenol (K_{ow} = 30.9), 1,4-dichlorobenzene (K_{ow} = 2455), and pentachlorophenol (K_{ow} = 132,000). Ionized pentachlorophenol or pentachlorophenate has an octanol-water partition coefficient of 7700 at pH 7.

Experimental methodology was developed to eliminate test compound removal from aqueous solution by the stripping and bio-oxidation mechanisms. Stripping was controlled by conducting experiments in a specially designed zero headspace reactor and the bio-oxidation mechanism was eliminated by inactivating the biomass via a lyophilization/heat treatment procedure. Studies were conducted comparing sorption results using the nonviable biomass and an active biomass culture. These studies were conducted using 1,4-dichlorobenzene and pentachlorophenol. These are relatively recalcitrant compounds. Results of these studies indicate close agreement between sorption results using nonviable and active microbial cultures.

Sorption kinetics were determined to be very fast for all three compounds with minimum first order sorption kinetic rate constants of 75.6, 37.8, and 8.2 hr^{-1} determined for phenol, 1,4-dichlorobenzene, and pentachlorophenol, respectively. Tests with phenol and 1,4-dichlorobenzene indicate that equilibrium was reached in less than 15 minutes. The sorption kinetic rate constants reported should be considered to be minimal values; significantly higher rate constants might be obtained as experimental methodology is improved to reduce the time required to acquire and process a sample at the first time point.

Pentachlorophenol sorption appears to follow a two-step process, with a very rapid initial sorption step and subsequent slower approach to equilibrium.

A linear relationship between the distribution coefficient and the octanol-water partition coefficient was developed for the three compounds. The distribution coefficient may be defined as:

$$K_{ba} = C_s/C_{ae}$$

where:

K_{ba} = sorption distribution coefficient from the aqueous to biomass compartments (L/g solids),
C_s = equilibrium compound loading on biological solids (μg/g), and
C_{ae} = equilibrium concentration of compound in aqueous phase (μg/L).

Results of this analysis are shown in Fig. 16. The following equation was developed by a linear regression of data shown in Fig. 16:

$$\text{Log}\ (K_{ba}) = -2.97 + 0.66\ [\text{Log}\ (K_{ow})] \qquad (r=0.95)$$

An equation has been proposed to predict the aqueous-biomass distribution coefficient (K_{ba}) as a function of the octanol-water partition coefficient and the lipid fraction in the biomass. This equation is based upon the hypothesis that sorption occurs into the internal lipids of the biomass.[58] The proposed equation is of the form:

$$K_{ba} = \frac{K_{ow}\ f_L}{\rho_L}$$

where:

f_L = lipid weight fraction of biomass, and
ρ_L = density of lipids (g/L).

Predicted versus observed K_{ba} values are presented in Fig. 17. Predicted values of K_{ba} assume that lipid weight fraction of biomass is 0.2, which is within the range reported in the literature.[58] As shown in Fig. 17, very close agreement is demonstrated between observed and predicted values of K_{ba} at low concentrations; however, observed K_{ba} values for 1,4-dichlorobenzene are significantly below predicted values at high aqueous phase concentrations. This effect may be due to an sorption saturation effect at high aqueous phase concentrations. Similar saturation effects were observed for pentachlorophenol in studies conducted in continuous reactors (Section 10).

BACKGROUND

There were two primary objectives of this study. The first was to develop an experimental method in which the bio-oxidation and stripping mechanisms would be controlled and the passive sorption of substrate from the aqueous compartment could be studied. The second was to use data collected by this method to test and further develop hypotheses with regard to mathematical equations describing the equilibria and kinetics of substrate sorption. The existence of well-understood mathematical equations describing sorption may serve as the basis for prediction of the sorption mechanism of organics in activated sludge treatment systems.

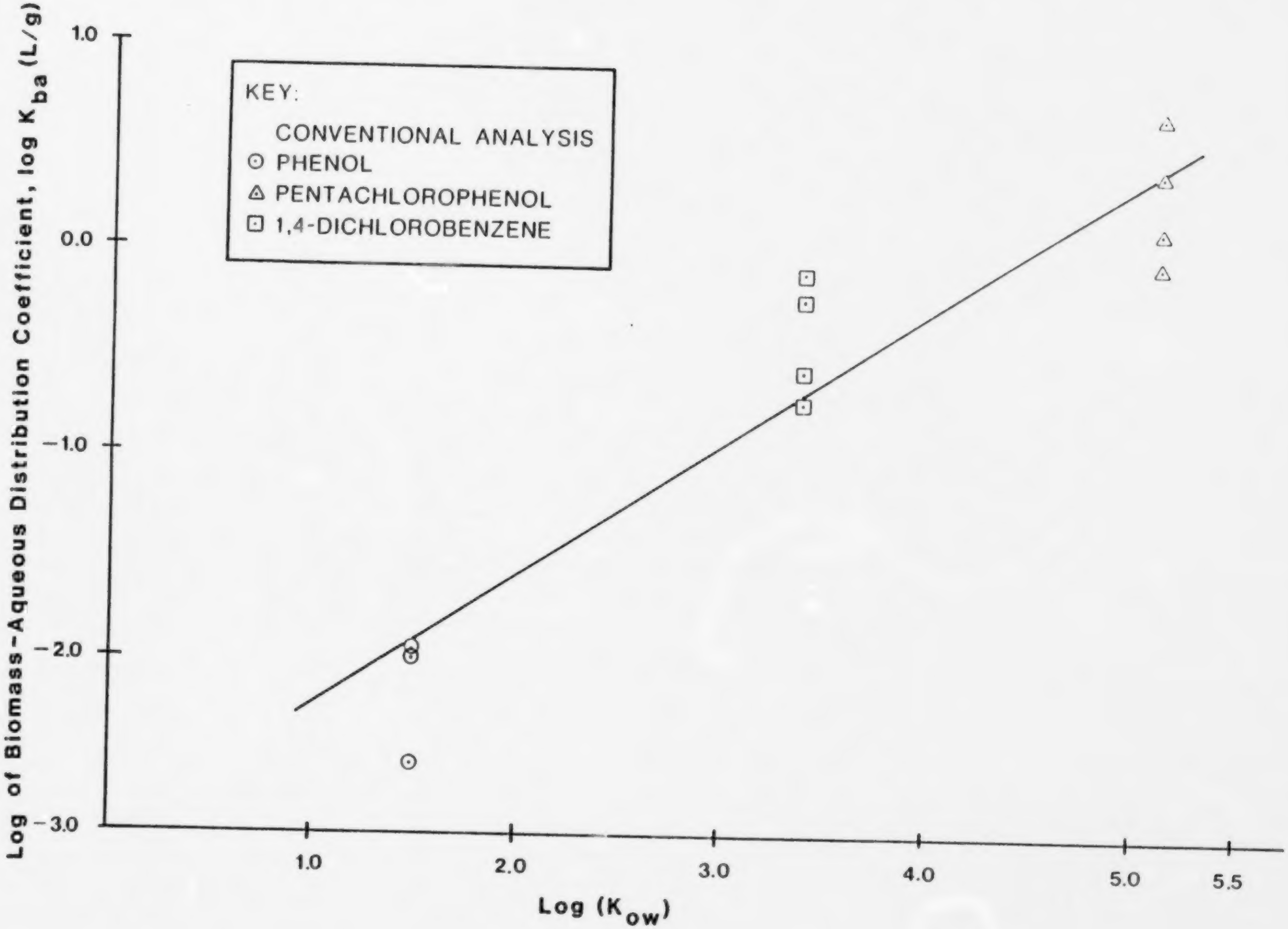

FIG. 16. LOG BIOMASS - AQUEOUS DISTRIBUTION COEFFICIENT VERSUS LOG OCTANOL - WATER PARTITION COEFFICIENT

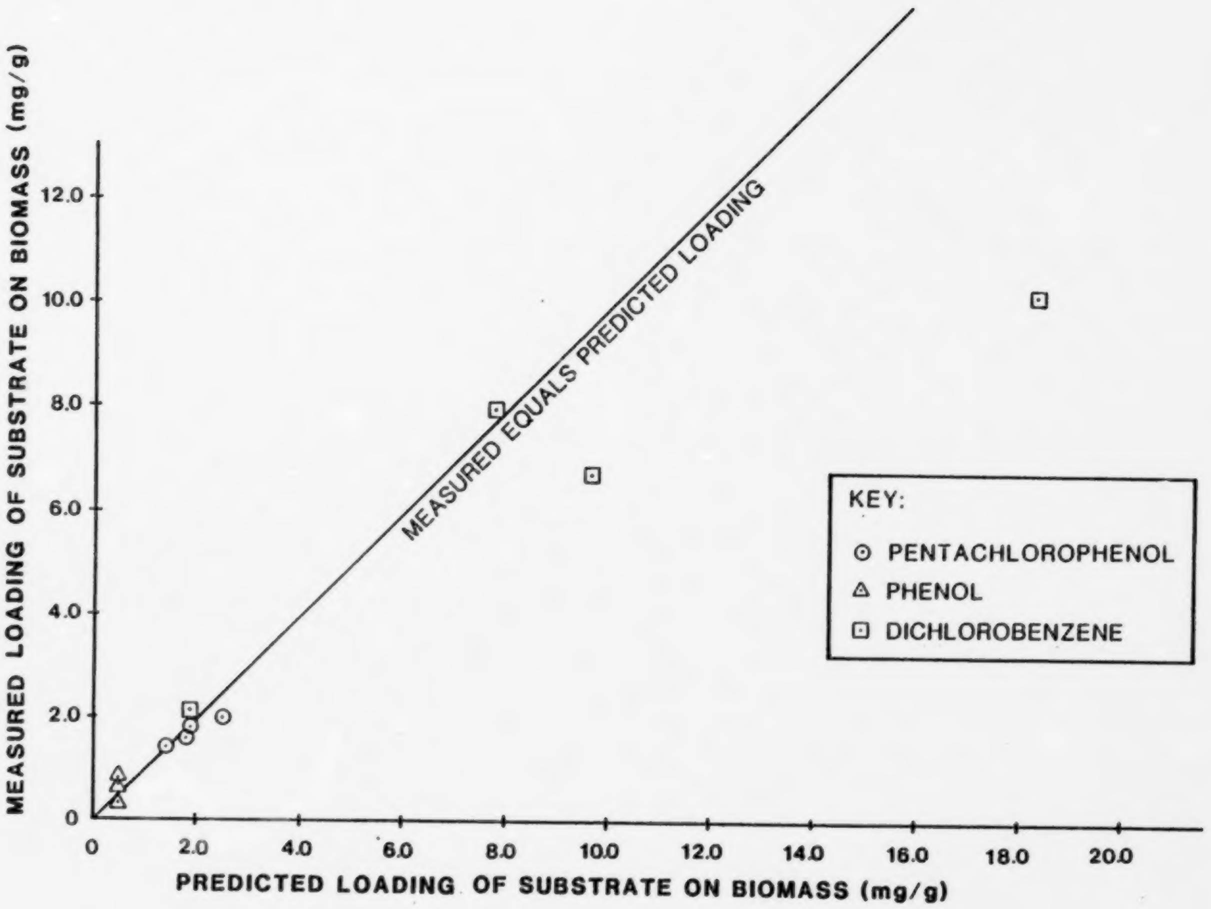

FIG. 17. MEASURED VERSUS PREDICTED (USING OCTANOL - WATER PARTITION COEFFICIENT) LOADING OF SUBSTRATE ON BIOMASS

A review of the mathematics of sorption is presented by Karickhoff.[15] He points out that in sufficiently dilute systems, typical of most environmental situations, sorption isotherms (plots of equilibrium substrate loading on solids vs. equilibrium aqueous phase substrate concentration) approach linearity. For hydrophobic compounds the term, "sufficiently dilute," corresponds to less than one half the solute water solubility or below 10^{-5} mole/L. Karickhoff reports that others have indicated the slopes of the linear isotherms can be predicted from the octanol-water partition coefficient. He also suggests that sorption/desorption kinetics reflect a diffusion controlled process.

Work in this area by Matter-Muller, Gujer, Giger, and Stumm[6] used pilot plant activated sludge processes to treat municipal sewage. For the compounds tetrachloroethylene, 1,2,3-trichlorobenzene, 1,2,4-trichlorobenzene and 1,2,3,4-tetrachlorobenzene, a formula was presented for the prediction of solids loading using the octanol-water partition coefficient.

Further work in this area by Steen and Karickhoff[10] and others as summarized by Baughman and Paris,[14] establishes that for various compounds, a relationship between the octanol-water partition coefficient and the solids loading does exist. In general, however, previous work has isolated the sorption pathway through selection of compounds with low stripping or biodegradative properties, or by use of substrate material balances.

In order to develop accurate data for strippable and biodegradable compounds using the octanol-water partition coefficient as a predictor, this study attempted to control the bio-oxidation and stripping mechanisms, allowing the measurement of the sorption mechanism. As described more fully later in this section, this control and measurement was achieved using a variable volume closed reactor and nonviable biomass. As reported by Baughman and Paris,[14] previous work indicated that substrate sorption to biological solids is largely unaltered by killing the organisms.

While previous work has given preliminary evidence that the octanol-water partition coefficient can be used as an equilibrium predictor for solids loading, a well-understood rationale and supporting conceptual model for this relationship does not exist. In addition, the prediction of end-point (equilibrium) concentration and sorption kinetics are not clearly understood. A major step in the development of a predictive fate method for sorption would be the clarification of the rationales for solids loading, equilibrium concentration, and kinetics.

As noted by Baughman and Paris,[14] it is not understood whether bioaccumulation occurs through adsorption or absorption, active transport, or passive transport. The proposed equations (discussed later) are based on the concept of cellular lipids, concentrated primarily in the cell surface, sorbing organics to some concentration (greater than the organic concentration in the aqueous phase) and the rate process being controlled by the octanol-water partition coefficient and lipid concentration. According to this hypothesis, the concentration of lipids in the biomass is an important parameter in the prediction of endpoint concentration, loading, and kinetics. The normalization of the partition coefficient to lipid content has been studied by others.[15] However, more data are needed to support a conceptual model.

It is noted that if this hypothesis is correct, the term "sorption" is incorrect for describing this process. Partitioning of the substrate between an aqueous compartment and a lipid compartment is an "extraction" process. However, for clarity and ease of understanding and because this hypothesis remains unproven, the term "sorption" will be used throughout this report.

MATERIALS AND METHODS

Process Equipment

Sorption experiments were performed in a variable volume closed reactor. Figures 18 and 19 present a diagram of the reactor and the overall system. Liquid volume changes in the reactor during sampling are compensated by movement of a piston activated by compressed air. The reactor can contain 9 L with allowable sampling of up to 4 L. Samples are removed from the reactor through a plug valve near the middle of the reactor body. Sample contents in the reactor are agitated with a variable speed mixer. The mixer motor is a Cole Parmer Model 4555GJ with a speed range varying from 0 to 130 rpm. Complete mixing of the reactor contents is aided by a set of four baffles situated in the upper half of the reactor body. The flanged neck of the reactor is sealed with an O-ring and glass reaction kettle cover combination. A U-clamp holds the glass cover to the reactor flange. The glass cover contains five Ace® threaded openings. The larger central opening allows the stirring rod to be sealed by an Ace Trubore bearing assembly. The four smaller threaded openings are around the larger opening. A bleed valve, a thermowell, and a manometer can be attached to these openings. The additional port can be used as needed.

Building air or bottled compressed air is controlled by a 0 to 8 psig regulator. The regulator is connected to the body of the reactor below the piston. The piston can be moved up the body of the reactor by 1 to 3 psig. Two polyurethane Parker Poly-Pak seals on the piston seal the driver air from the reactor contents. A 4-psig pressure relief valve prevents overpressuring of the reactor contents.

An inlet port is located 90° axially from the sample port. Piping is attached to the inlet port, allowing feed to flow from a glass 12-L mixing vessel, through a Micro Pump Model 8110 variable speed gear pump, through a 2-micron in-line filter and a 3-way ball valve. The ball valve can be positioned to allow flow to enter the reactor or to be diverted to a drain.

The feed mixing vessel has an Ace threaded opening at the top through which a stirring rod is inserted. The agitator motor is a Cole Parmer Model 4555GJ with a speed range varying from 0 to 130 rpm. An Ace Trubore bearing seals the stirring rod. Four smaller Ace threaded openings are

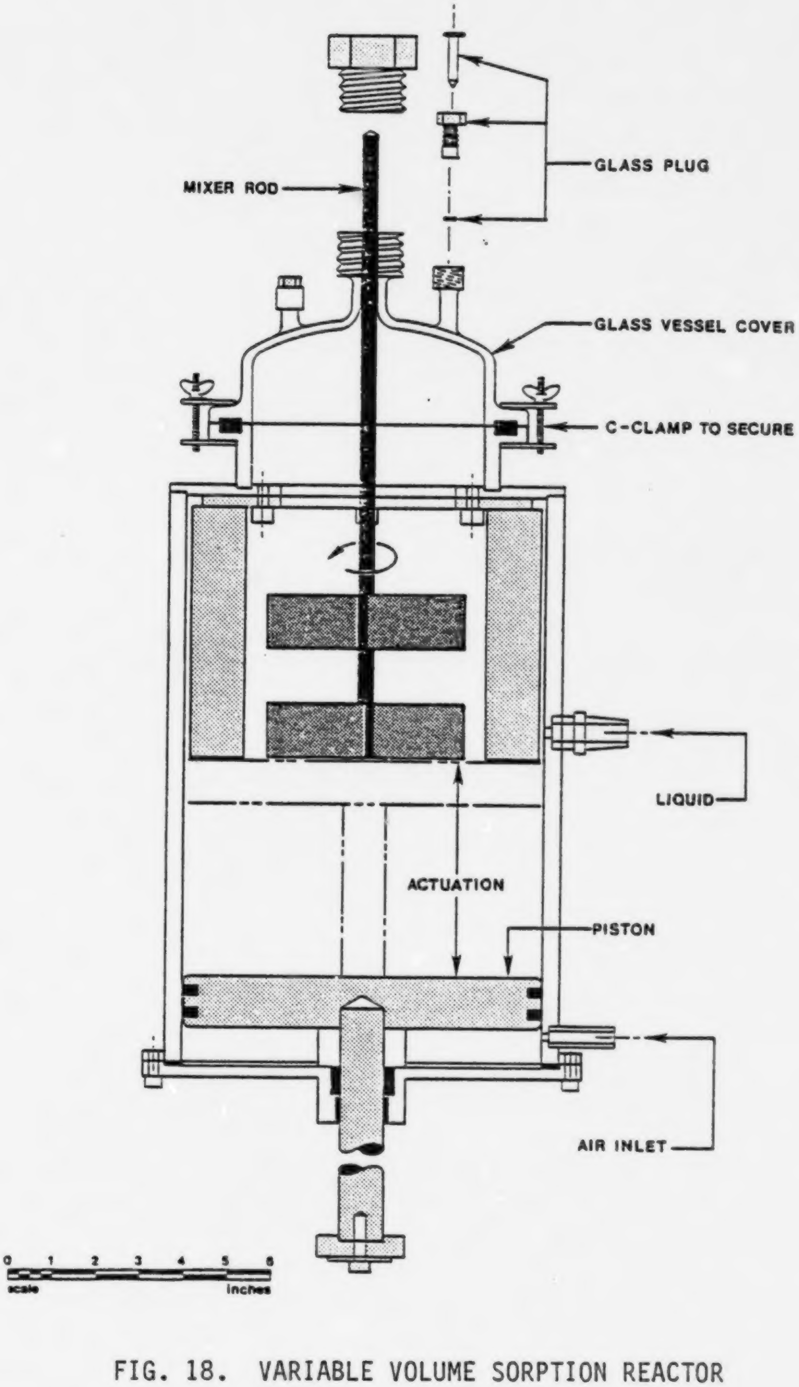
GLASS PLUG
MIXER ROD
GLASS VESSEL COVER
C-CLAMP TO SECURE
LIQUID
ACTUATION
PISTON
AIR INLET
0 1 2 3 4 5 6
scale
inches

FIG. 18. VARIABLE VOLUME SORPTION REACTOR

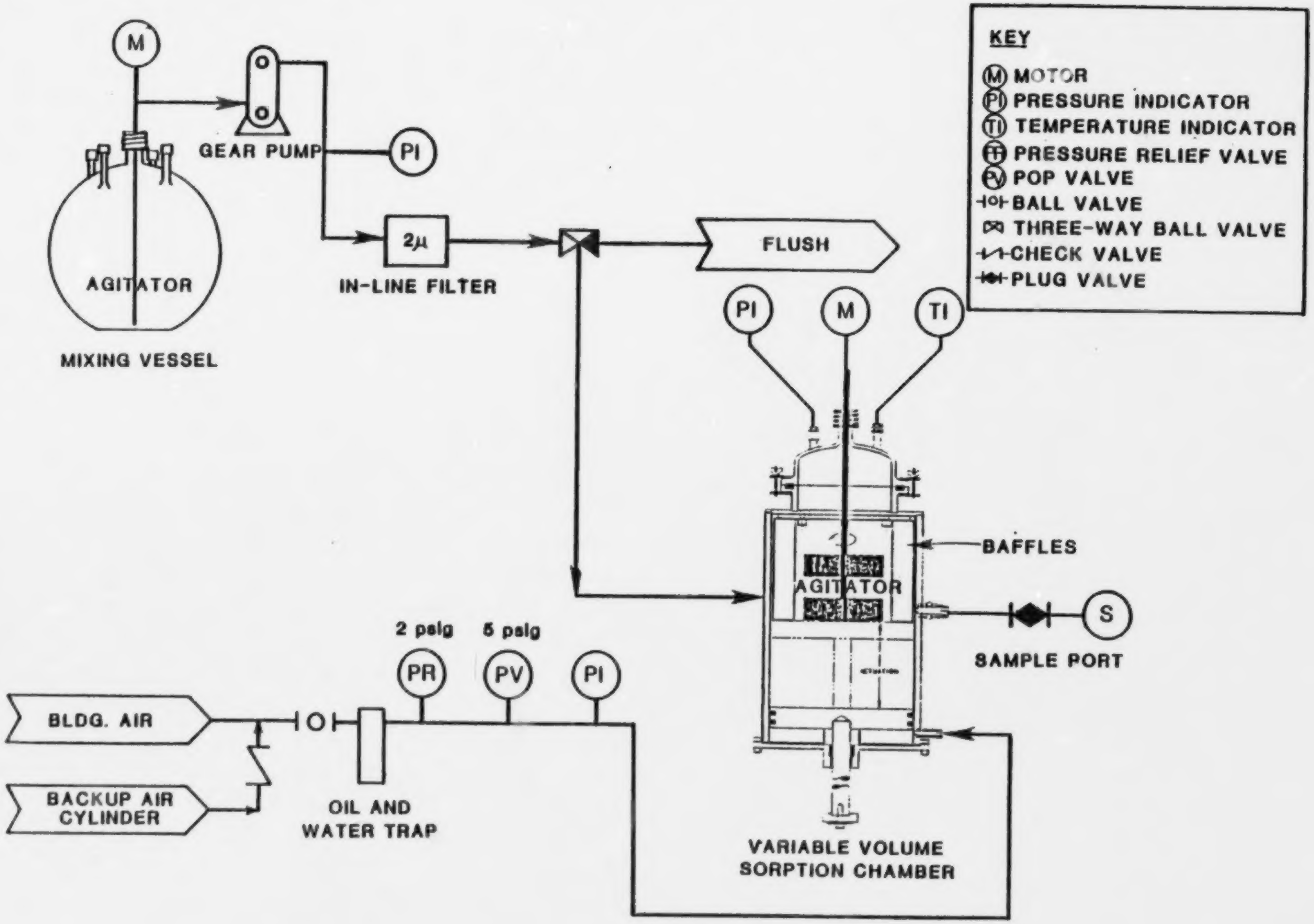
KEY
M MOTOR
PI PRESSURE INDICATOR
TI TEMPERATURE INDICATOR
PR PRESSURE RELIEF VALVE
PV POP VALVE
BALL VALVE
THREE-WAY BALL VALVE
CHECK VALVE
PLUG VALVE
M
AGITATOR
MIXING VESSEL
GEAR PUMP
PI
2μ
IN-LINE FILTER
FLUSH
PI
M
TI
BAFFLES
AGITATOR
S
SAMPLE PORT
2 psig
5 psig
PR
PV
PI
BLDG. AIR
BACKUP AIR CYLINDER
OIL AND WATER TRAP
VARIABLE VOLUME SORPTION CHAMBER

FIG. 19. SCHEMATIC OF SORPTION SYSTEM

located on a radius around the larger central threaded opening. These openings can be used to connect a nitrogen blanket line or an input line to the mixing vessel.

All wetted parts of the sorption system are either 316 stainless steel, glass, or Teflon.

Experimental Procedure

Sorption experiments SP-1 through SP-11 were carried out by contacting biological solids in a sealed variable volume batch reactor with an aqueous solution containing the test compound and withdrawing samples at discrete time increments. Experimental variables include the initial concentrations of the substrate and biological solids, the condition of the biological solids (viable, inactivated with formaldehyde, inactivated via lyophilization/heat treatment) and temperature effects. Experimental procedures also varied in the manner of introducing feed to the reactor. The specific method used to prepare the biomass and the mode of feed introduction to the reactor will be detailed by experiment. Table 40 shows the feed and biomass used in each experiment.

TABLE 40. EXPERIMENTAL PARAMETERS FOR SORPTION EXPERIMENTS

Experiment number	Feed	Biomass condition
SP-1	1,4-Dichlorobenzene	Formaldehyde-treated
SP-2	1,4-Dichlorobenzene	Viable
SP-3	Phenol	Lyophilized-dry heated
SP-4	Phenol	Lyophilized-dry heated
SP-5	Phenol and radiolabeled phenol	Lyophilized-dry heated
SP-6[a]	Phenol and radiolabeled phenol	Lyophilized-dry heated, cooled to 4°C
SP-7	Pentachlorophenol and radiolabeled pentachlorophenol	Lyophilized-dry heated
SP-8	Pentachlorophenol	Viable
SP-9	Pentachlorophenol and radiolabeled pentachlorophenol	Lyophilized-dry heated
SP-10	1,4-Dichlorobenzene and radiolabeled 1,4-dichlorobenzene	Lyophilized-dry heated
SP-11	Pentachlorophenol, 1,4-dichlorobenzene and radiolabeled 1,4-dichlorobenzene	Lyophilized-dry heated

[a] Less severe mixing conditions.

For phenol experiments SP-5 and SP-6, 12 liters of deionized water was transferred into a 12-liter glass mixing vessel. A measured weight of phenol was then quantitatively transferred to the mixing vessel and the mixing vessel contents were mixed for approximately 24 hr. After mixing, samples were taken from the mixing vessel and analyzed by liquid chromatography to determine the phenol feed concentration.

A similar procedure was followed in phenol experiments SP-3 and SP-4 except that the feed solution was prepared in multiple 12-liter volumetric flasks instead of a single mixing vessel.

For the 1,4-dichlorobenzene and pentachlorophenol experiments (SP-1, 2, 7, 8, 9, 10, and 11), 12 liters of deionized water were transferred into the glass mixing vessel. The test compound was weighed, dissolved in 12 mL of HPLC methanol, and quantitatively transferred to the mixing vessel with a 5-mL Luer-tip syringe fitted with a 13-cm needle. The syringe needle was introduced through one of the small threaded openings at the top of the vessel. The mixing vessel contents were then stirred for approximately 24 hr. Feed sample solutions containing 1,4-dichlorobenzene were analyzed by GC and sample solutions containing pentachlorophenol were analyzed by LC to determine the concentration of the test compound in the feed solution.

Experiments SP-5, 6, 7, 9, 10, and 11 were performed using ^{14}C labeled test compounds. Solutions of ^{14}C labeled test compounds were prepared by the same methods used to prepare the non-radiolabeled test compounds. Initial concentrations of ^{14}C labeled compounds were assayed by methods described in Appendix B.

Sorption experiment 1 (SP-1, 1,4-dichlorobenzene) used formaldehyde-treated biomass prepared by the method detailed in Section 7. Sorption experiments 2 and 8 (SP-1, 1,4-dichlorobenzene and SP-8, pentachlorophenol) used live biomass from biomass acclimation reactors 1 and 2. Sorption experiments SP-3, phenol; SP-4, phenol; SP-5, phenol; SP-6, phenol; SP-7, pentachlorophenol; SP-9, pentachlorophenol; SP-10, 1,4-dichlorobenzene; and SP-11, 1,4-dichlorobenzene/pentachlorophenol combined run, used biomass prepared by the lyophilization/dry heat treatment method detailed in Section 7.

The freeze-dried biomass was rehydrated in a 50-L glass battery jar. Approximately 40 L of deionized water was added to the battery jar and then mixed with a high speed (0 to 12,000 rpm) Chemineer Bench Scale Model shear mixer for 3 min at 5000 rpm. The solids were then allowed to settle overnight. Supernatant was drawn off by siphon and the solids left in the battery jar. Forty liters of deionized water was added to the jar and the solids were mixed by hand with a spatula. Solids were allowed to settle for 1 hr before the supernatant was siphoned off. Solids were further concentrated by centrifugation at 1000 rpm for 10 min in an IEC model Centra 7R centrifuge.

Live biomass used in experiments SP-2 and 8 was pumped from the biomass acclimation reactors into a 50-L glass battery jar and allowed to settle. Supernatant was removed by siphon. The solids were further concentrated by centrifugation.

Before adding solids and feed to the reactor, process equipment was prepared for the experiment. The air pressure delivery system was connected to the reactor. The piston was positioned so that the reactor volume was at a maximum. The stirring rod was polished with steel wool at the bearing contact points. The stirrer bearing was lubricated with inert bearing grease and fitted to the stirring rod. Before feed addition, feed samples were taken. Except for experiments SP-10 and SP-11, the inlet port to the reactor was closed. In experiments SP-10 and SP-11, the inlet port was piped according to the procedure outlined in the process equipment discussion in this section. A control sample of the centrifuged solids to be added to the reactor was taken, and the control wet sample weight was determined. The solid control sample was dried at 105°C for several hours so that the percent free water weight and percent dry weight of the sample could be determined. These percentages and the wet solids weight were used to calculate the dry weight of solids and the free water actually added to the reactor.

Following the preparatory steps, a solution containing solids was added to the reactor. The weight of wet biomass was recorded. In experiments SP-1 through SP-9 the feed containing the test compound was added to the reactor through the neck at the top of the reactor. The glass cover was then fit into place and clamped to the reactor flange. The stirring rod was attached to the variable speed mixing motor by a flexible rubber hose connection. The mixer was turned on and the air pressure adjusted to approximately 2 psig. The specific power range input to the system by the agitator was 4.3 to 7.7 hp/1000 ft^3 for all runs except SP-6. The range for SP-6 was 0.6 to 1.0 hp/1000 ft^3. These values are given as a range corresponding to the volume of the reactor from 9 L to 5 L. Runs other than SP-6 experienced much higher mixing intensities than those found in full-scale plants. However, results here should represent basic sorption rates minimizing liquid film diffusive limitations. Further work was planned at lower mixing intensities but was limited by timing and budget considerations. Air was allowed to escape from the top of the reactor through a bleed valve attached to the reactor cover. Once the reactor was full of liquid, the bleed valve was closed and sampling commenced. A leak check was performed to ensure that the system was liquid tight.

Chemicals and Reagents

The chemicals used in sorption experiments are as follows:

- Methanol, acetonitrile, acetone, and methylene chloride were HPLC grade
- Phenol, 1,4-dichlorobenzene and pentachlorophenol were ACS grade or better; purities were >99%
- Toluene and p-dioxane were scintillation grade
- Hexane was distilled in glass, non-spectra grade
- Formaldehyde was a 37% pharmaceutical grade solution
- Carbon-14 labeled phenol and 1,4-dichlorobenzene were >99% purity
- Carbon-14 labeled pentachlorophenol purity data are not available; (Analytical tests, conducted according to methods listed in Appendix B, showed no impurities)

Analytical Methods

Detailed methods for the analysis of phenol, pentachlorophenol, and 1,4-dichlorobenzene in the sorption study experiments can be found in Appendix B. The methods outlined were chosen or developed to provide valid analytical data for each particular compound and experimental matrix. When possible, the samples were analyzed immediately after sampling to minimize losses, otherwise the samples were suitably preserved for later analysis.

Stock standards were prepared by weighing the appropriate pure compound into a volumetric flask and diluting to volume with reagent grade water or the appropriate solvent for water insoluble compounds.

Secondary standards for analysis were prepared to bracket the range of the samples within the linear range of the instrument. The secondary standards were prepared by volumetric dilution of the stock standards with a matrix solution appropriate to the analytical method.

Sampling Methods

Forty-mL samples were obtained in 50-mL conical tip centrifuge tubes from the sampling port in the side of the reactor at 3 to 10 min intervals following loading of the reactor and the start of mixing. The samples were capped, weighed, and immediately centrifuged at 3000 rpm for 5 min. Twenty-five mL of the supernatant was removed with a pipette to provide the aqueous sample. This supernatant was filtered through a 0.45 μm filter to remove additional biological solids before analysis. An aliquot of the filtered sample was diluted with the required solvents or water to provide the appropriate matrix for analysis.

The remainder of the supernatant was removed from the centrifuge tube sample using a Pasteur pipet. The volume of the residual wet solids was recorded and the weight of the wet biomass sample recorded. Five mL of a solvent was added to the wet biomass (basic methanol for phenol and pentachlorophenol and methylene chloride for dichlorobenzene) and allowed to remain in contact for approximately 12-16 hr. The sample was then centrifuged again at 3000 rpm for 5 min and the supernatant liquid removed by pipet. The extracts containing phenol and pentachlorophenol were acidified with 0.3 N HCl. The acidified extract was centrifuged and filtered through a 0.45 μm filter prior to analysis. The filtered sample was then diluted with solvent or water to obtain the appropriate matrix for analysis.

Mathematical Methods

A linear relationship between substrate aqueous phase concentration and substrate loading on biological solids at equilibrium may be given as follows:

$$K_{ba} = C_s/C_{ae} \qquad \text{Eqn. 46}$$

where:

K_{ba} = sorption distribution coefficient (L/g solids),
C_s = equilibrium compound loading on biological solids (μg/g), and
C_{ae} = equilibrium concentration of compound in aqueous phase (μg/L).

An equation has been proposed to predict the distribution coefficient (K_{ba}) as a function of the octanol water partition coefficient and the lipid fraction in the biomass. This equation is based upon the theory that sorption occurs on the internal lipids of the biomass.[59] The proposed equation is of the form:

$$K_{ba} = \frac{K_{ow} f_L}{\rho_L} \qquad \text{Eqn. 47}$$

where:

f_L = lipid weight fraction of biomass and
ρ_L = density of lipids (g/L).

Equations 46 and 47 can be used to write an equilibrium material balance for a batch reactor if the following conditions are satisfied:

- Sorption is the only substrate removal mechanism present
- There is no growth of biological solids in the reactor
- There is no substrate sorbed to the biological solids at time zero
- There is no saturation effect (maximum solids loading) at the concentrations studied

This equation has the form:

$$C_{ao} V_a = \left(C_{ae} V_a\right) + \left(C_s X V_a \cdot \frac{1g}{1000 \text{ mg}}\right) \qquad \text{Eqn. 48}$$

where:

V_a = aqueous phase volume (L),
C_{ae} = equilibrium aqueous phase substrate concentration (μg/L),
X = solids (biomass) concentration (mg/L, dry basis),
C_{ao} = initial aqueous phase substrate concentration (μg/L), and
C_s = solids loading on biomass (μg/g).

Equations 46, 47, and 48 can be combined to predict the equilibrium aqueous phase concentration in a batch reactor as follows:

$$C_{ae} = \frac{C_{ao}}{K_{ow} (X f_L / 1000 \rho_L) + 1} \qquad \text{Eqn. 49}$$

The equilibrium loading on solids is

$$C_s = \frac{C_{ao}}{(X/1000) + (\rho_L/K_{ow}\ f_L)} \qquad \text{Eqn. 50}$$

Equations 49 and 50 were used in the experimental design phase of this work to predict equilibrium conditions for a set of given experimental conditions and to predict sample sizes required for analytical work. For example, a compound such as phenol with a low K_{ow} value was expected to exhibit a very low loading onto biological solids, hence a relatively large sample of solids must be extracted to recover a quantifiable mass of phenol. Initial equilibrium estimates were made using various values of K_{ow} and a weight fraction of lipids in solids equal to 0.2. It was assumed that the lipid density equaled 900 g/L. A solid concentration of 5000 mg/L was also assumed. This was close to the actual solids concentration run. A range of starting concentrations was used to generate endpoint (equilibrium) concentration vs. loading curves. It was from these curves that upper and lower K_{ow} values were selected for use in targeting specific compounds for this study.

Later in this study, the above equations were used to predict aqueous phase concentrations and loading values at equilibrium at specific operating conditions in each experiment. A range of equilibrium conditions were bracketed by varying the assumed weight fraction of lipids in solids between 0.05 and 0.2.

A proposed equation describing the kinetics of sorption is based on standard mass transfer type kinetic relationships, first-order in biomass concentration, and using the difference in the equilibrium loading and the instantaneous loading as the mass-transfer driving force. The equilibrium loading is stated in terms of the equilibrium aqueous phase concentration by substitution of equations 46 and 47.

Kinetic Equation, Batch Reactor

$$-\frac{dC_a^{sp}}{dt} = K_a^{sp}\left[\frac{f_L K_{ow}\ C_{ae}}{1000\ \rho_L} - C_{sb}\right] \qquad \text{Eqn. 51}$$

where:

$-\frac{dC_a^{sp}}{dt}$ = instantaneous rate of disappearance of aqueous phase substrate concentration due to sorption,

K_a^{sp} = substrate biomass sorption rate constant (hr^{-1}),

C_a, C_{ae} = aqueous phase substrate concentration and equilibrium concentration (μg/L), and

C_{sb} = sorbed solute loading in the biomass (mg solute/mg solids).

Common practice in chemical reactor design is to apply batch system kinetic rate constants to the design of continuous reactors. (See

Levenspiel[60] for more information in this area.) The premise in this study was to collect data from a batch system, determine the sorption rate constant ($K_a{}^{ad}$) for various compounds, apply that rate constant in the above equation, and thus predict continuous system kinetics.

RESULTS AND DISCUSSION

Calculation of Sorption Concentration and Loading Data

Caution was taken in these experiments to attempt to correct the calculated concentration and loading variables for the effect of dissolved substrate in the extracellular biomass water. That is, any dissolved substrate existing between cells but not partitioning within the cell was corrected for by a series of calculations. This calculational procedure is generally described as follows:

- $C_s = \dfrac{(C_{ao} - C_{ae})\ (V_F + V_{ecw})}{B_d}$ Eqn. 52
- $C_{ao} = \dfrac{F_{ao}\ V_F}{V_F + V_{ecw}}$ Eqn. 53
- $V_{ecw} = B_w\ f_{ab}/\rho_{ecw}$ Eqn. 54
- $V^S_{ecw} = S_w f_{ab}/\rho_{ecw}$ Eqn. 55
- $f_{ab} = \dfrac{S_w - (S_d/R)}{S_w}$ Eqn. 56

Where:

C_s = the equilibrium or endpoint loading of substrate on the biomass (mg/g, dry basis),

C_{ao} = calculated initial aqueous phase concentration (mg/L),

C_{ae} = measured endpoint or equilibrium aqueous phase concentration (mg/L),

F_{ao} = measured concentration of the aqueous reactor feed (mg/L),

V_F = measured volume of the aqueous reactor feed (L),

V_{ecw}, V_{ecw}^{s} = calculated volume of extracellular water for total biomass, and sample, respectively (L),

B_w = measured weight of wet biomass in the experiment (g),

B_d = measured weight of dry biomass in the experiment (g),

f_{ab} = calculated weight fraction of water in wet biomass,

S_w = measured wet weight of solids in sample (mg),

S_d = measured dry weight of solids in sample (mg), and

R = cellular dry weight/cellular weight with no extracellular water, assumed to be 0.2.

Correction for the effects of extracellular water requires at least one assumption and a series of calculations where error is propagated. However, direct measurement of extracellular water is difficult because of the membraneous structure of the cell. Water can transport in or out of the cell during the analysis process. It may be possible to use high-speed centrifugation to generate a cell pellet with minimal extracellular water but the effect of pressure on the cellular water would require investigation.

A method where the cells are passed through a hydrophillic solvent in a centrifuge to remove extracellular water may be a practical method if it can be shown that extraction of substrates into the solvent is minimal or can be controlled.

The issue of extracellular water becomes very important if large amounts of substrate reside in the water and are counted as being sorbed. This may be expected always for low K_{ow} compounds and for high K_{ow} compounds if the aqueous concentrations exceed a saturation value. In these cases, failure to correct for extracellular water effects is likely to result in highly variable and incorrect biomass sorption loadings and distribution coefficients.

The starting concentration is a calculated value because of the impracticality of attaining a measured initial value with sorption occurring so rapidly. It should be noted that all calculations are strongly affected by this calculated starting concentration and therefore more error is expected to be propagated than if the starting concentrations could be accurately measured.

Phenol Experiments

Figures 20 through 23 show the experimental sorption rate data for the four phenol runs. In every case, observed aqueous phase equilibrium con-

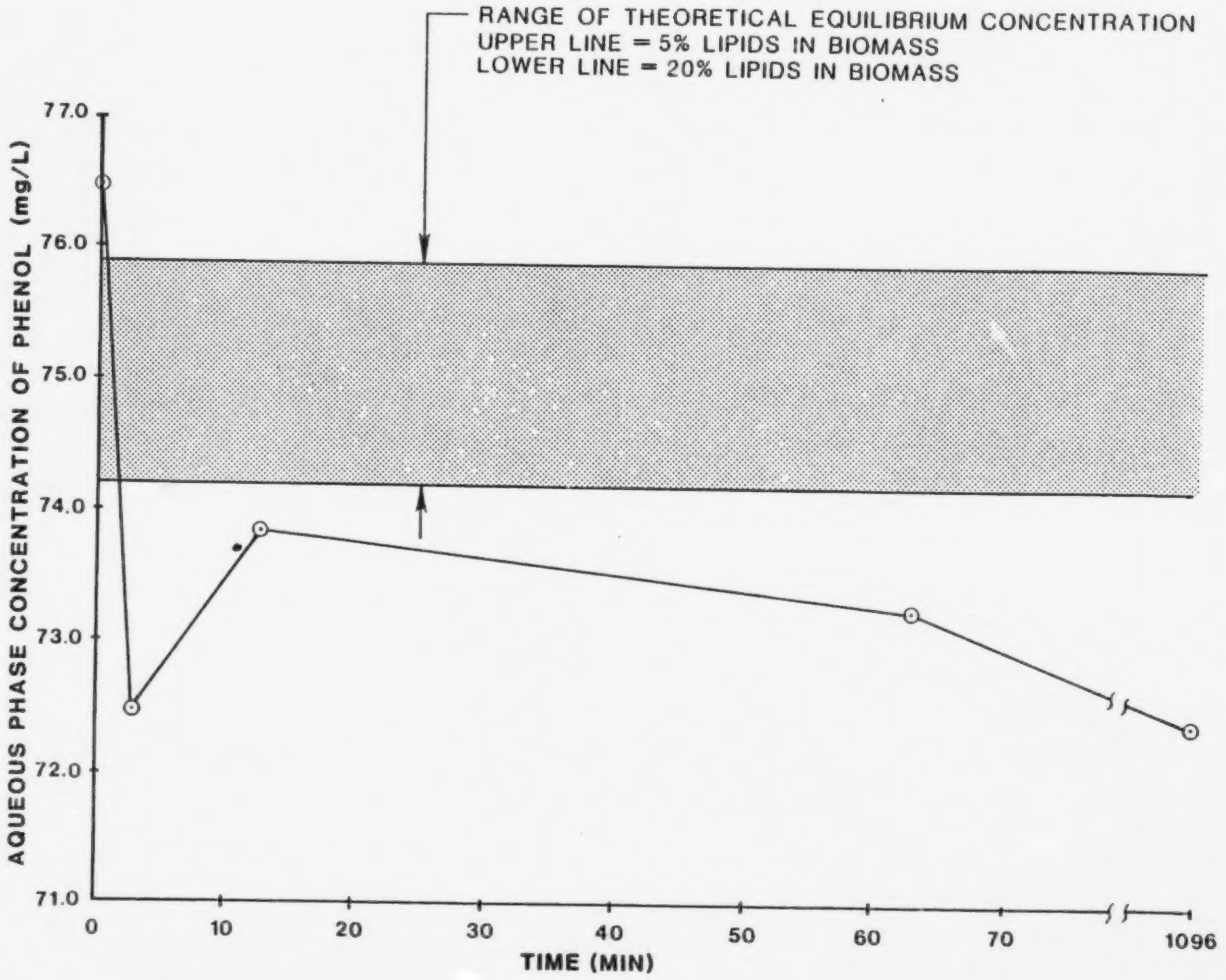

FIG. 20. SORPTION CURVE, PHENOL RUN SP-3: LYOPHILIZED BIOMASS

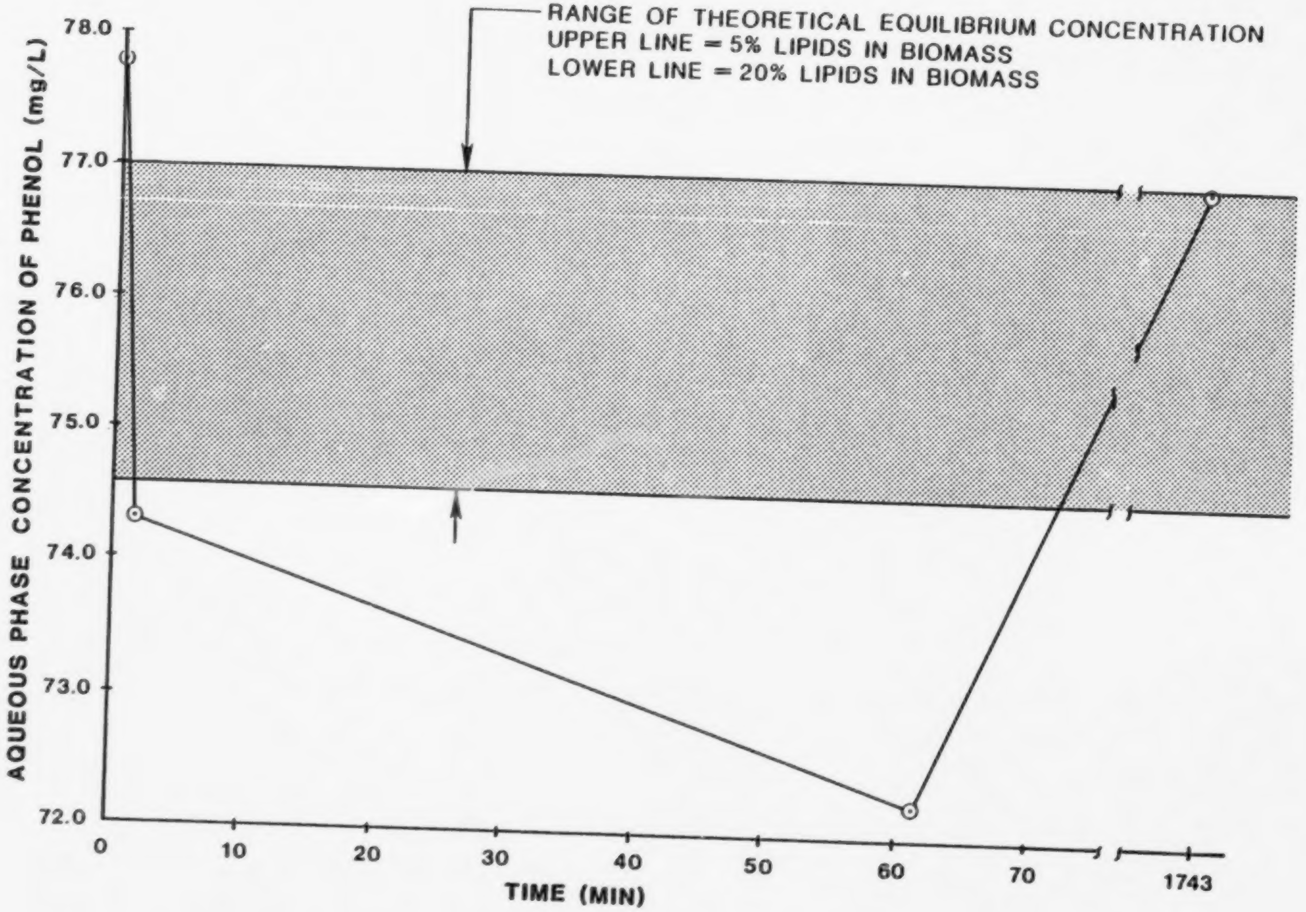

FIG. 21. SORPTION CURVE, PHENOL RUN SP-4: LYOPHILIZED BIOMASS

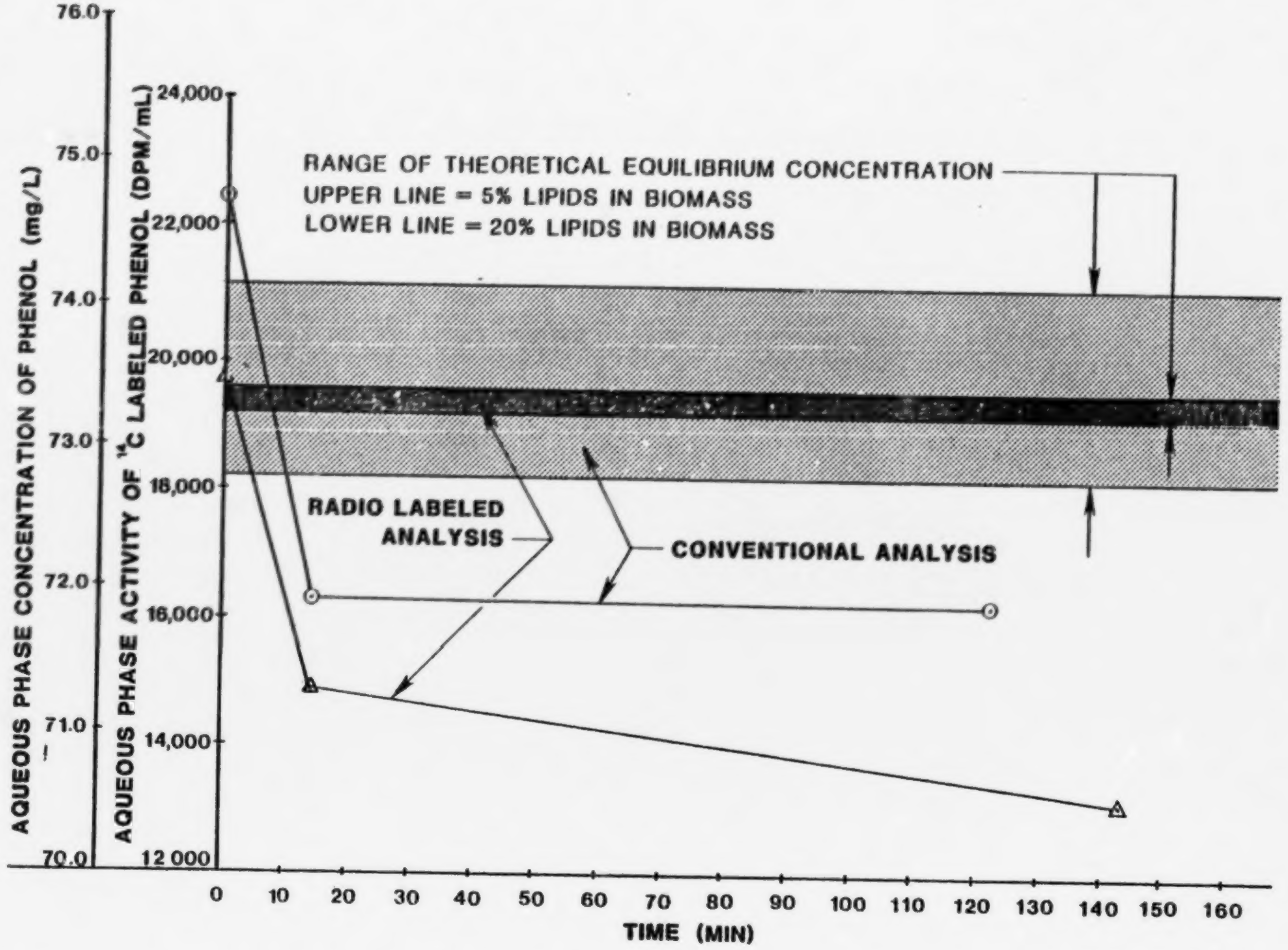

FIG. 22. SORPTION CURVE, PHENOL AND RADIOLABELED PHENOL RUN SP-5: LYOPHILIZED BIOMASS

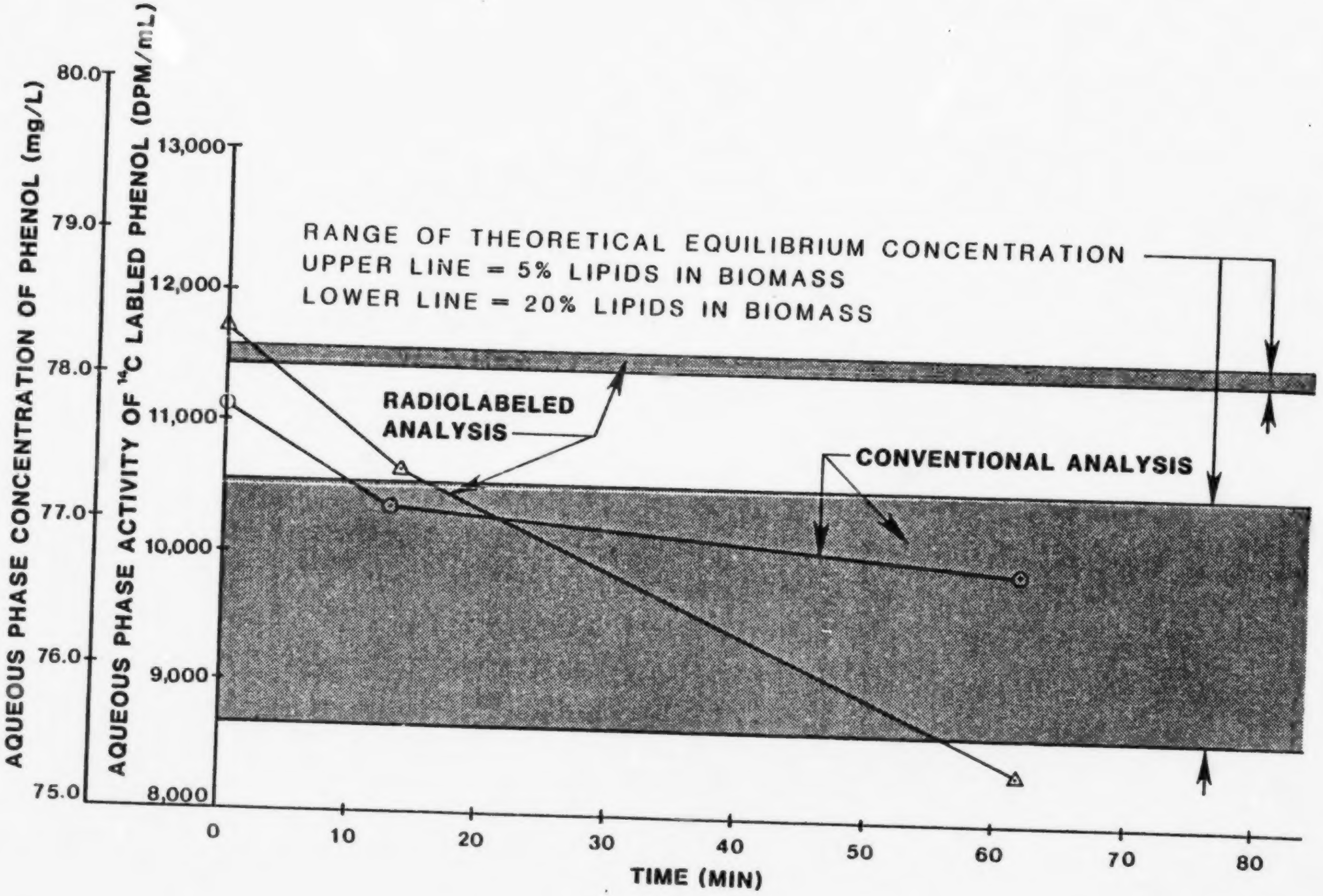

FIG. 23. SORPTION CURVE, PHENOL AND RADIOLABELED PHENOL, RUN SP-6: LYOPHILIZED BIOMASS

centration fell within or below the equilibrium concentration range predicted using Eqn. 49. Table 41 and Fig. 24 present equilibrium aqueous phase concentration and loading data for the four phenol runs. The loadings are in good agreement with the predicted values, with the exception of run SP-6, which was performed at a reduced temperature. The poor material balance for run SP-6 may be explained by a very small concentration change (2 mg/L). This small concentration change magnifies the effect of analytical errors, as reflected in the material balance.

TABLE 41. PHENOL SORPTION RESULTS

Parameter	Run SP-3	Run SP-4	Run SP-5[a]	Run SP-6[a,b]
Starting concentration (mg/L)[c]	76.5	77.8	74.7	77.8
Endpoint concentration (mg/L)	73.0	73.3	71.8	76.8
Predicted endpoint concentration[d] (mg/L)	74.2	74.6	72.8	75.7
Loading[e] (mg phenol/g dry biomass)	0.8	0.7	0.8	0.3
Predicted loading[d] (mg phenol/g dry biomass	0.5	0.5	0.5	0.5
Material balance closure (%)[f]	78	92	89	253
Biomass in reactor, dry basis (g)	29.5	47.7	29.7	27.8
Total aqueous volume in reactor (L)	6.5	7.6	7.9	7.0
Biomass condition	Lyophilized	Lyophilized	Lyophilized	Lyophilized

[a] Corresponding radiolabeled analysis found in Table 45.

[b] Cooled to 4°C.

[c] See Eqn. 53.

[d] Prediction based on 20% lipid content, using equations 49 and 50.

[e] See Eqn. 52.

[f] Material balance closure = $\frac{\text{compound analytically recovered from solid phase}}{\text{compound lost from aqueous phase}}$ X 100, numerator includes phenol recovered in extract plus phenol in extracellular solvent (see Appendix E).

As shown in Figs. 20 through 23, the initial sorption rate is very rapid and the aqueous phase concentration measured at the first time point is generally close to the final aqueous phase equilibrium concentration. Sorption kinetics may be more rapid than illustrated by these data, with calculated values of rate constants strongly dependent upon the minimum time required to process samples. A similar observation holds true for studies conducted with 1,4-dichlorobenzene.

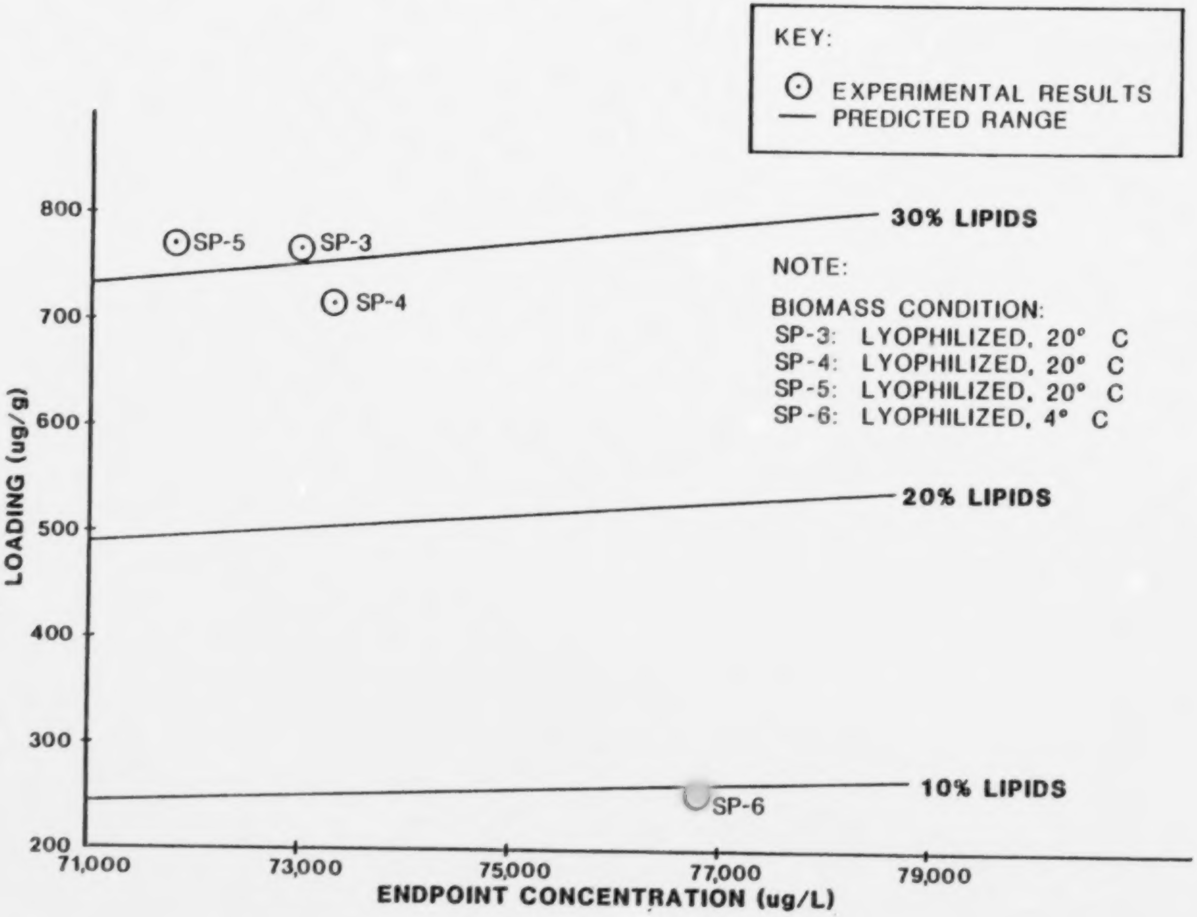

FIG. 24. LOADING VERSUS ENDPOINT CONCENTRATION, PHENOL

First order kinetic rate constants were calculated for data at the first time point using the following equation. Although empirical in nature, this equation assumes the exponential type decay kinetics that follows first order behavior on batch reactors.

$$K_a{}^{sp} = \frac{\ln (C_{ao} - C_{ae} / C_a - C_{ae})}{\Delta t} \qquad \text{Eqn. 57}$$

where:

$K_a{}^{sp}$ = first order sorption rate constant (hr^{-1}),

C_{ao} = initial test compound concentration in the aqueous phase (mg/L),

C_{ae} = equilibrium test compound concentration in aqueous phase (mg/L),

C_a = test compound concentration in aqueous phase at time t (mg/L), and

Δt = elapsed time (hr).

The rate constants for the phenol runs are shown in Table 42. The rate constant for Run SP-6, which was performed at a reduced temperature and lower specific power input, is lower than the other rate constants. However, as mentioned previously, analytical errors due to extremely low concentration changes may be a factor in the data from Run SP-6.

TABLE 42. MINIMUM SORPTION RATE VALUES, CONVENTIONAL ANALYSIS

		Sorption kinetic rate constant, $K_a{}^{sp}$ (hr^{-1})	
Compound	Run	1st step	2nd step
Phenol	SP-3	75.6	
Phenol	SP-4	33.2	
Phenol	SP-5	14.1	
Phenol	SP-6	5.3	
1,4-Dichlorobenzene	SP-1	7.2	
1,4-Dichlorobenzene	SP-2	10.8	
1,4-Dichlorobenzene	SP-10	24.7	
1,4-Dichlorobenzene	SP-11	37.8	
Pentachlorophenol	SP-7	1.4	
Pentachlorophenol	SP-8	7.0	
Pentachlorophenol	SP-9	3.5	0.03
Pentachlorophenol	SP-11	8.2	0.4

1,4-Dichlorobenzene Experiments

Figures 25 through 28 show the observed sorption data and compare actual and predicted equilibrium aqueous phase concentrations for the four 1,4-dichlorobenzene runs. Radiolabeled 1,4-dichlorobenzene was also used in runs SP-10 and SP-11. The observed equilibrium aqueous phase concentrations fall within or below the range predicted by Eqn. 49 in every case. Table 43 and Fig. 29 present equilibrium aqueous phase concentrations and loading data for the four conventional runs. The loadings are in good agreement between themselves and with the predicted values. The poor material balance closure for Run SP-11 may be explained by a small aqueous phase concentration change (10.0 mg/L) as compared to the other three runs. This small concentration change magnifies the effect of analytical errors as reflected in the material balance.

TABLE 43. 1,4-DICHLOROBENZENE SORPTION RESULTS

Parameter	Run SP-1	Run SP-2	Run SP-10[a]	Run SP-11[a]
Reactor starting[b] concentration (mg/L)	46.7	77.5	51.0	12.7
Endpoint concentration (mg/L)	26.6	54.2	13.9	2.7
Predicted endpoint concentration[c] (mg/L)	17.8	33.8	14.3	3.5
Loading[d] (mg DCB/g dry biomass)	6.7	9.8	7.9	2.7
Predicted loading[c] (mg DCB/g dry biomass)	9.7	18.4	7.8	1.9
Material balance closure (%)[e]	f	f	103	190
Reactor biomass, dry basis (g)	26.9	21.3	45.4	46.4
Total aqueous volume in reactor (L)	9.0	9.0	9.7	9.8
Biomass condition	Formaldehyde-treated	Viable	Lyophilized	Lyophilized

[a] Corresponding radiolabeled analysis found in Table 45.

[b] See Eqn. 53.

[c] Prediction based on 20% lipid content, using K_{ow} prediction equations.

[d] See Eqn. 52.

[e] Material balance closure = $\frac{\text{compound recovered from solid phase}}{\text{compound lost from aqueous phase}} \times 100$.

[f] Balance not closed. Insufficient data.

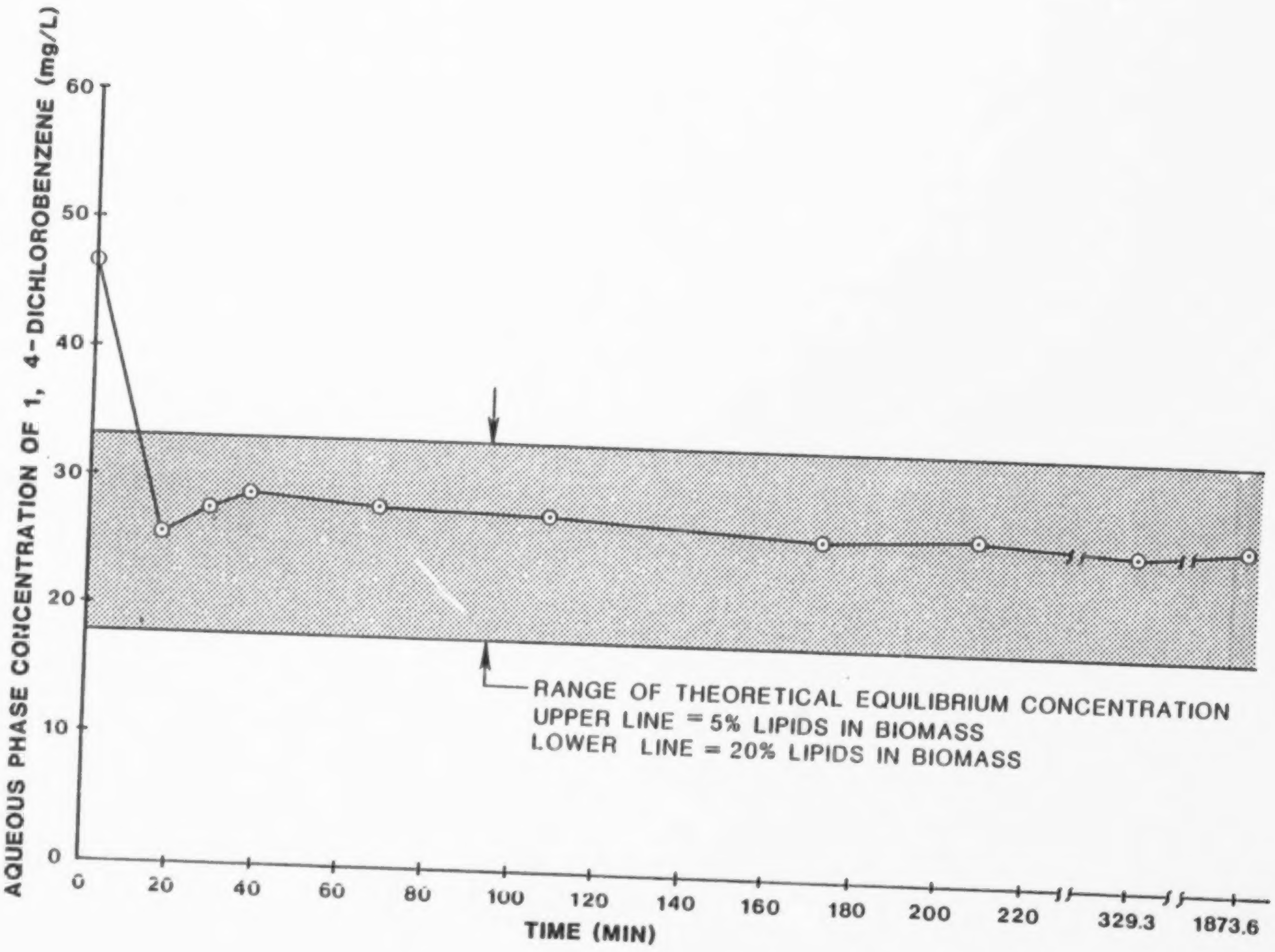

FIG. 25. SORPTION CURVE, 1, 4-DICHLOROBENZENE RUN SP-1: FORMALDEHYDE-TREATED BIOMASS

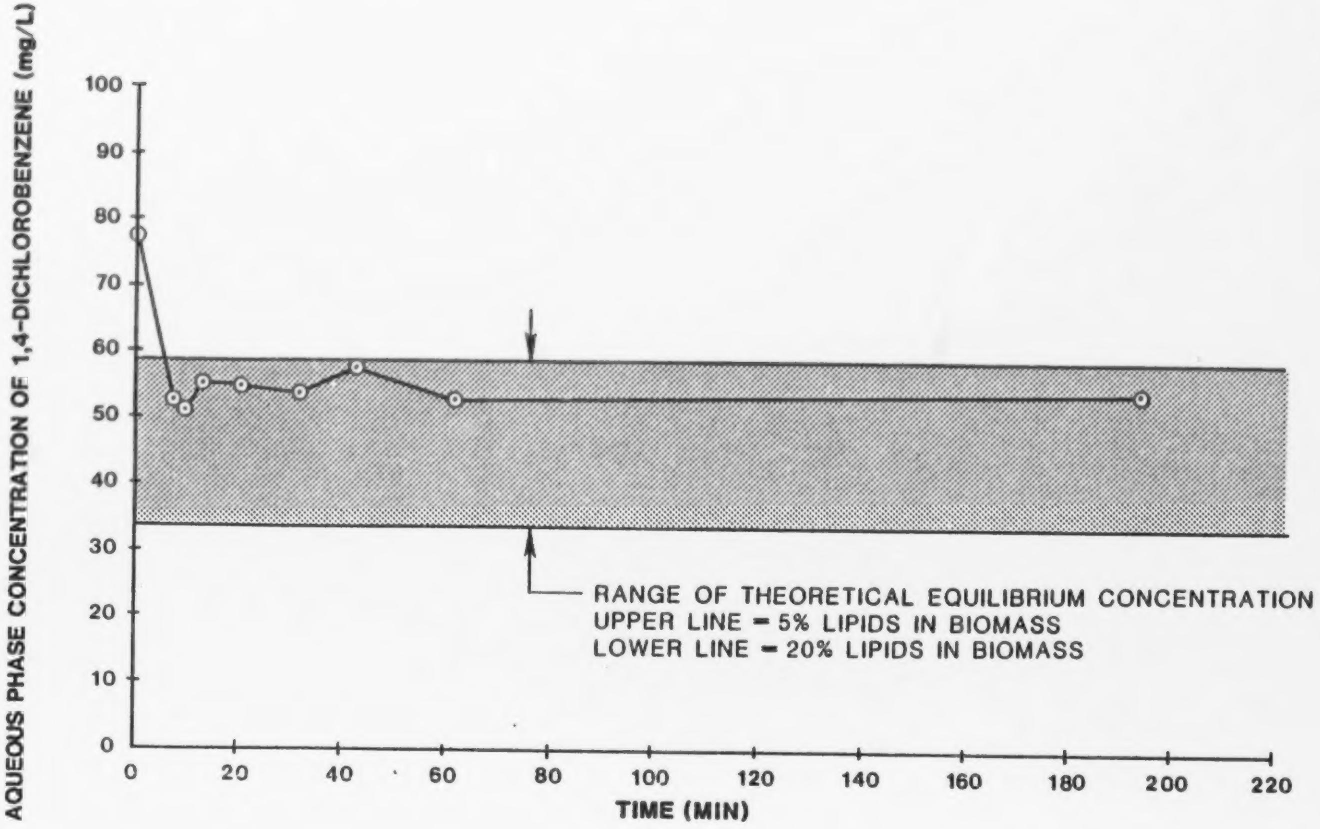

FIG. 26. SORPTION CURVE, 1,4-DICHLOROBENZENE RUN SP-2: VIABLE BIOMASS

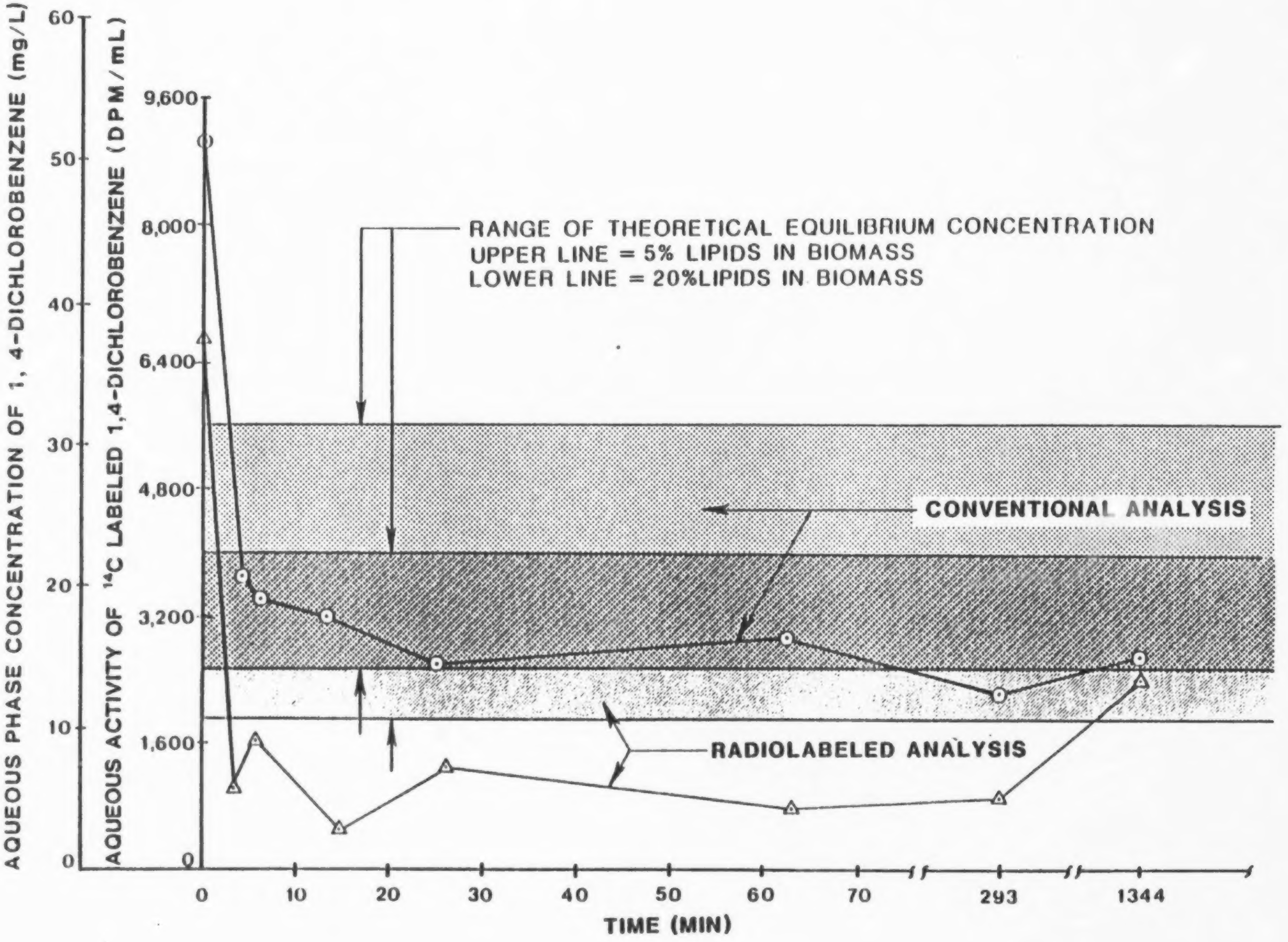

FIG. 27. SORPTION CURVE, 1,4-DICHLOROBENZENE RUN SP-10: LYOPHILIZED BIOMASS

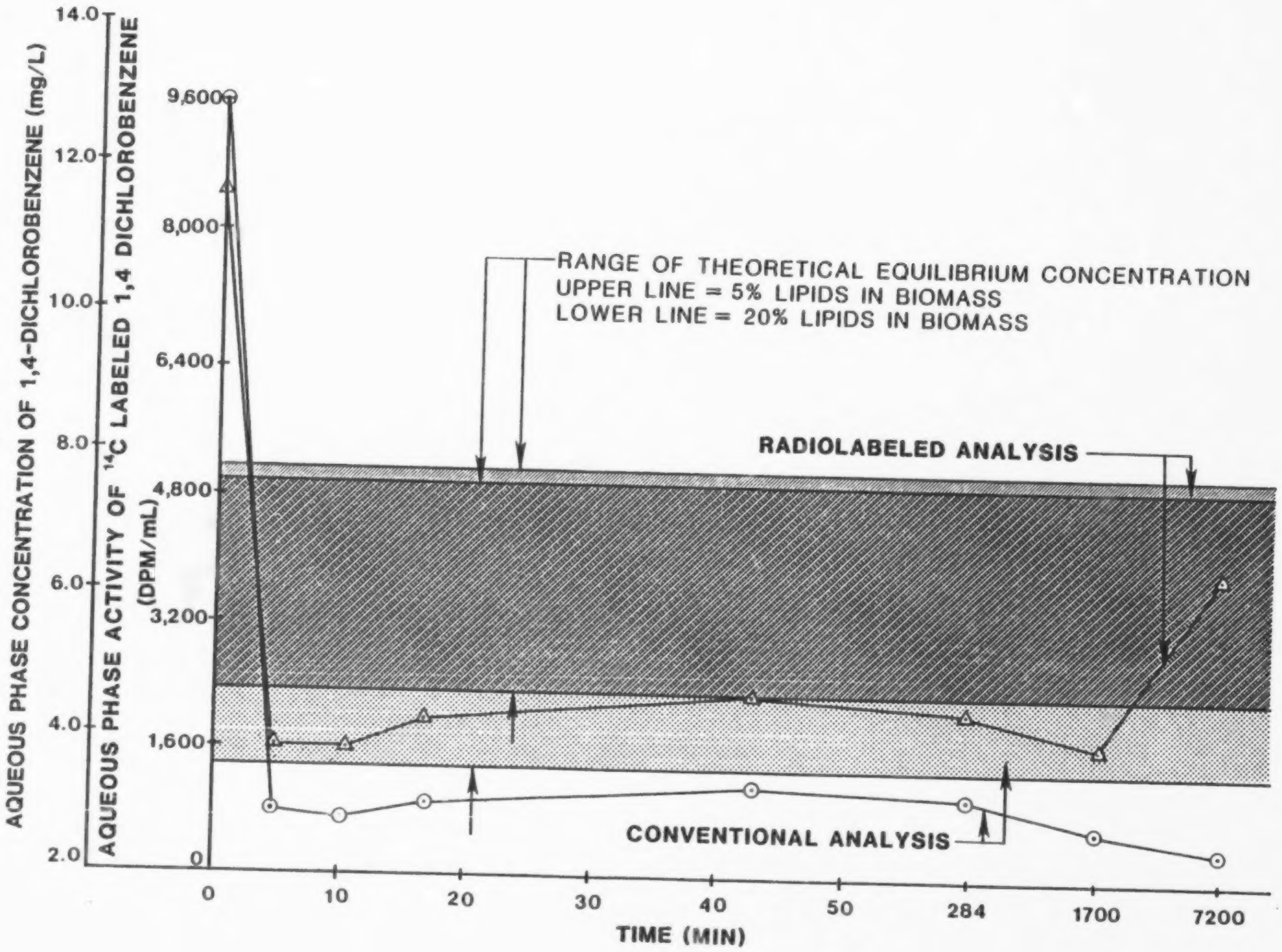

FIG. 28. SORPTION CURVE, 1,4-DICHLOROBENZENE RUN SP-11: LYOPHILIZED BIOMASS

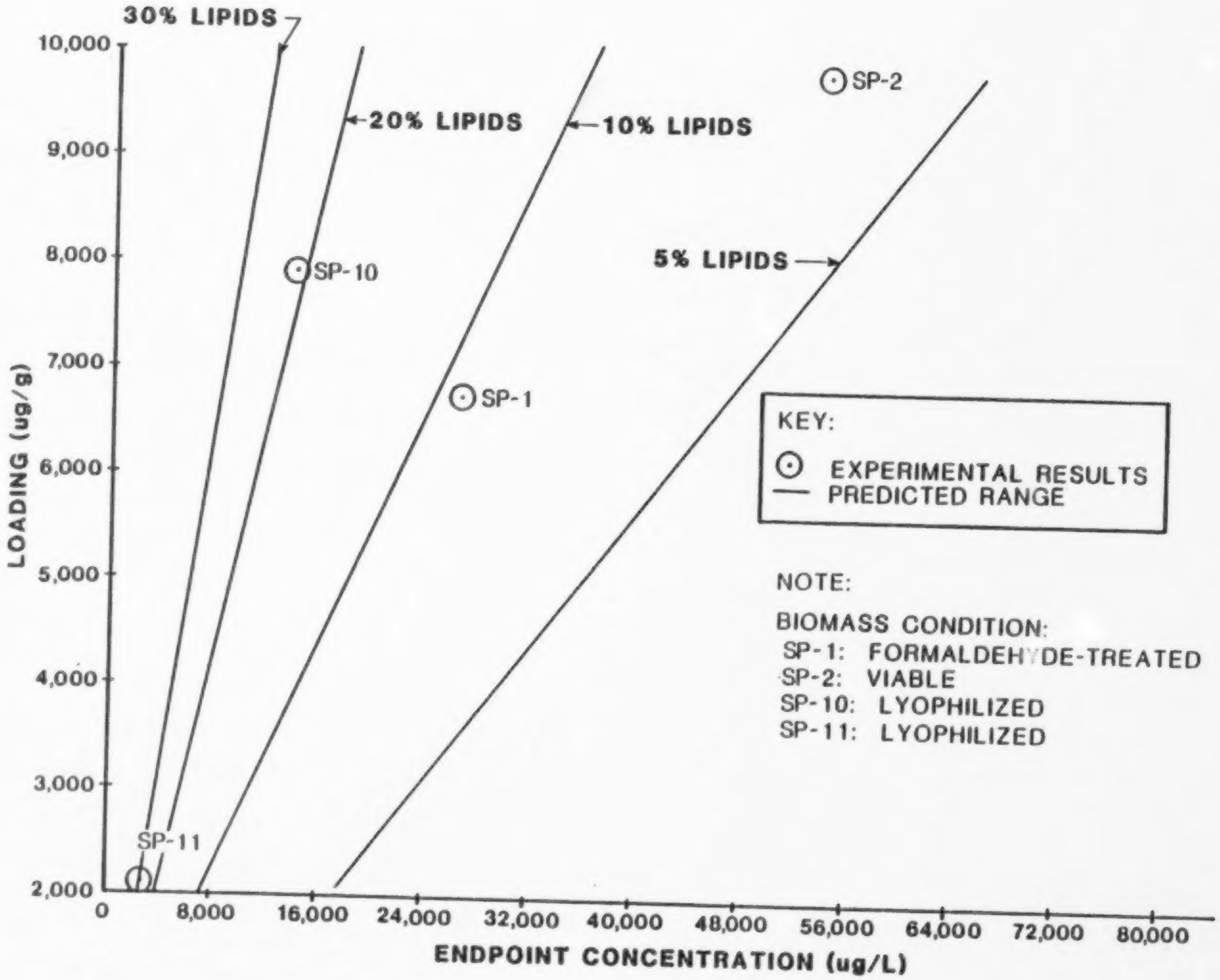

FIG. 29. LOADING VERSUS ENDPOINT CONCENTRATION, 1,4-DICHLOROBENZENE

The sorption rate constants for the four 1,4-dichlorobenzene experiments are shown in Table 18.

Pentachlorophenol Experiments

Figures 30 through 33 show the experimentally observed sorption rates and compare actual and predicted equilibrium concentrations for the four pentachlorophenol runs. Radiolabeled pentachlorophenol was also used in Runs SP-7 and SP-9.

When the lower value of 7700 is used for the K_{ow} in the prediction equation, the agreement between measured and predicted values for PCP is quite good. However, the K_{ow} value of 7700 is higher than that expected with PCP having a pK_a of 4.8. Even though the chemistry giving rise to this effect is elusive, the K_{ow} value of 7700 for pH 7 has been confirmed in two labs.[62,63]

Figure 34 shows that when results from the batch experiments described in this section are compared to results from continuous runs discussed in Section 10, the data from the continuous runs appear to show a saturation effect. This will be discussed more fully in Section 10.

Table 44 and Fig. 35 present equilibrium concentration and loading data for the four pentachlorophenol runs. The loadings show very good agreement between themselves and with the predicted values.

The wide apparent variance of the lipid content on Figs. 24, 29, and 35 might suggest that the lipid fraction of the biomass changes substantially from test to test depending on the type of biomass used -- lyophilized, formaldehyde-treated, or cold-viable. However, one must remember that the loading calculation is a function of the calculated starting concentration in the experiment and some variation from experiment to experiment can be expected. It is possible, however, that the lyophilization-dry heating process removes non-lipid volatile organic carbon from the biomass thus increasing the effective lipid concentration of the dry biomass. For the moment, the ability to establish an endpoint concentration and loading within the ranges seen in these experiments must be considered a success. Lipid concentration determinations can be developed in further work.

The sorption rate constants are summarized in Table 42. Runs SP-9 and SP-11 seem to exhibit a two-stage mechanism, a rapid stage followed by a much slower stage. Insufficient data were collected in runs SP-7 and SP-8 to support or contradict this suggestion. Extraction of biological solids contained impurities that interfered with the detection of pentachlorophenol when analyzed by liquid chromatography. Because of this problem, pentachlorophenol concentrations were not quantifiable in the biomass extracts. Further analytical development was precluded because of budget and timing constraints.

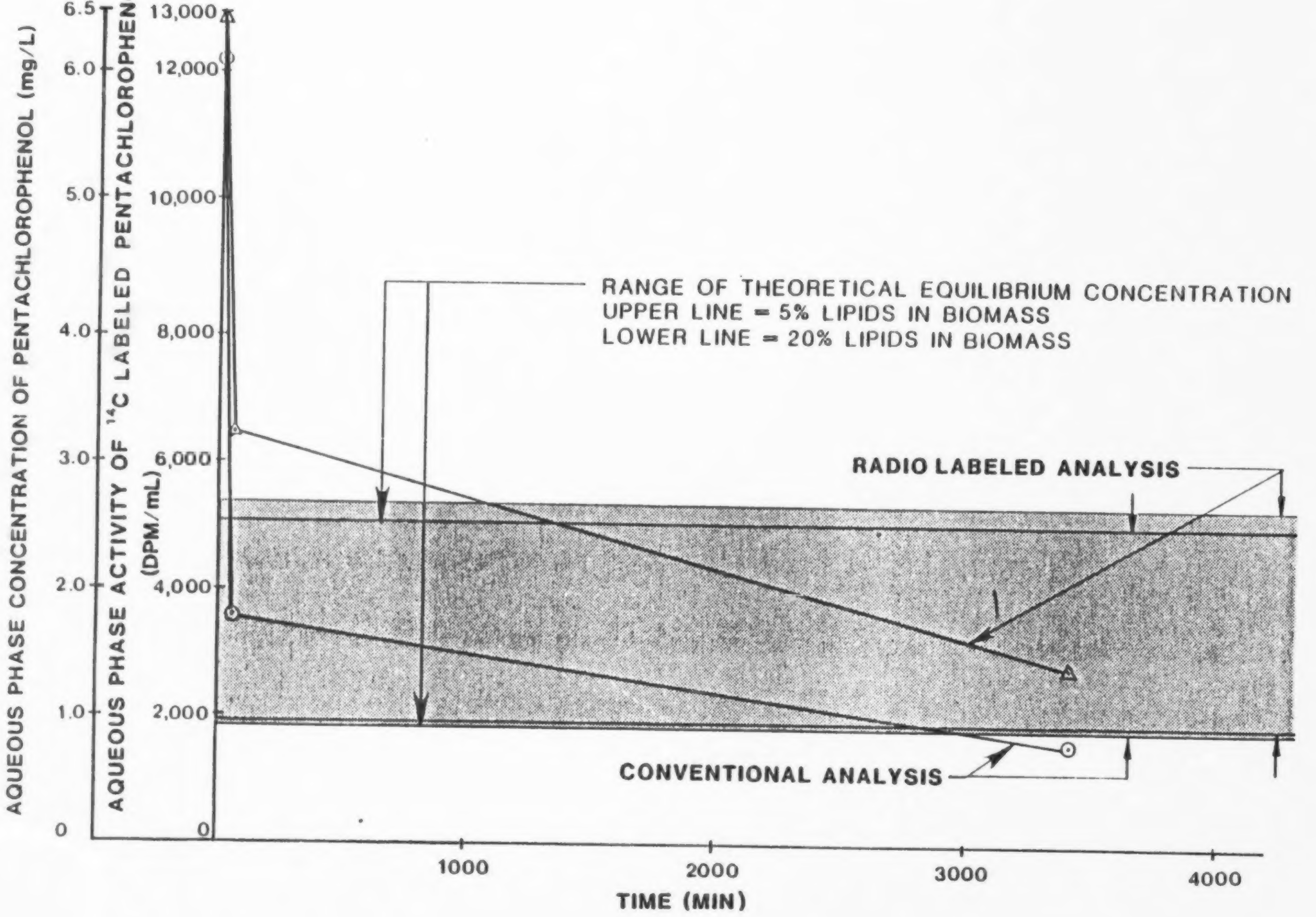

FIG. 30. SORPTION CURVE, PENTACHLOROPHENOL RUN SP-7: LYOPHILIZED BIOMASS

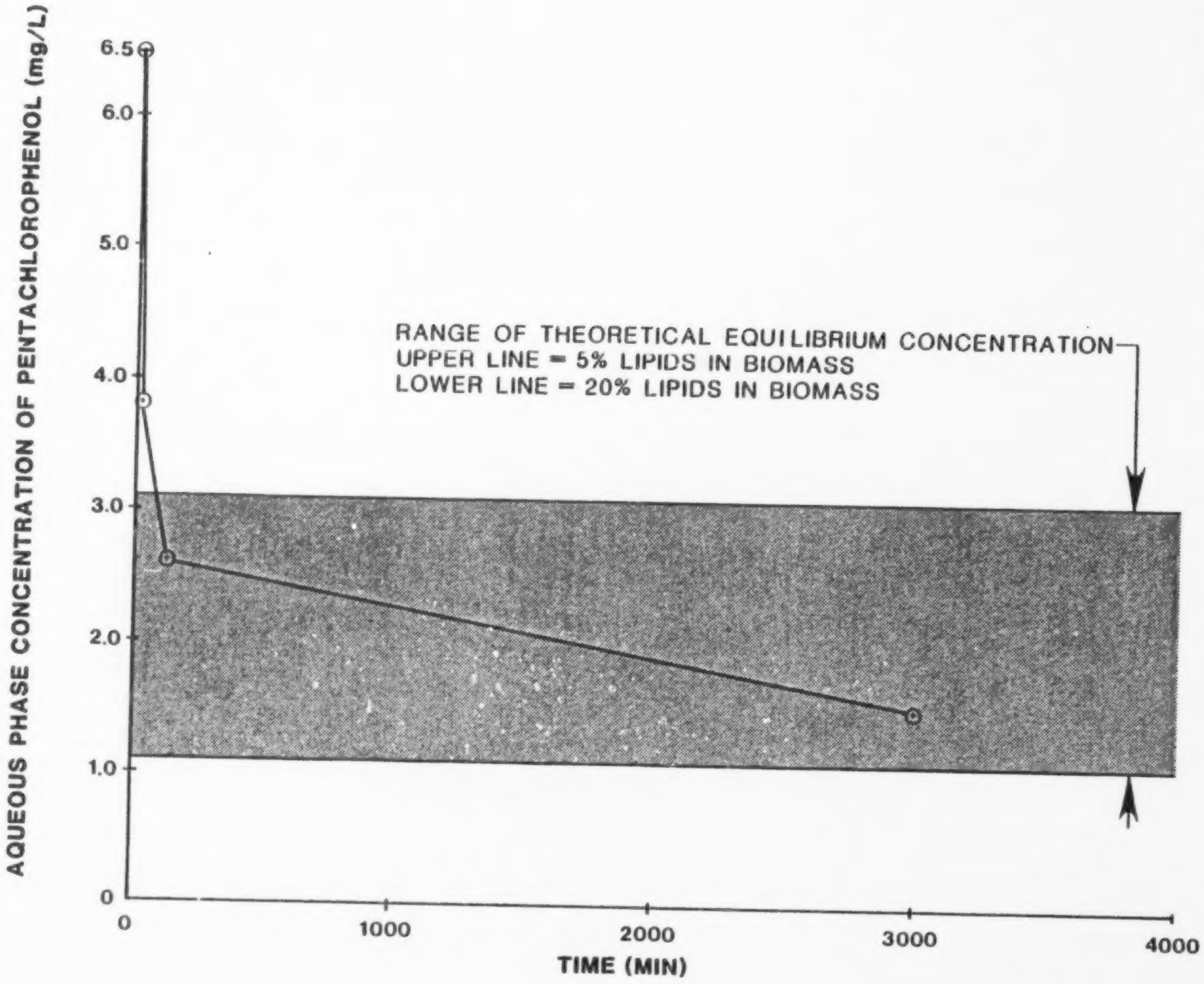

FIG. 31. SORPTION CURVE, PENTACHLOROPHENOL RUN SP-8: VIABLE BIOMASS

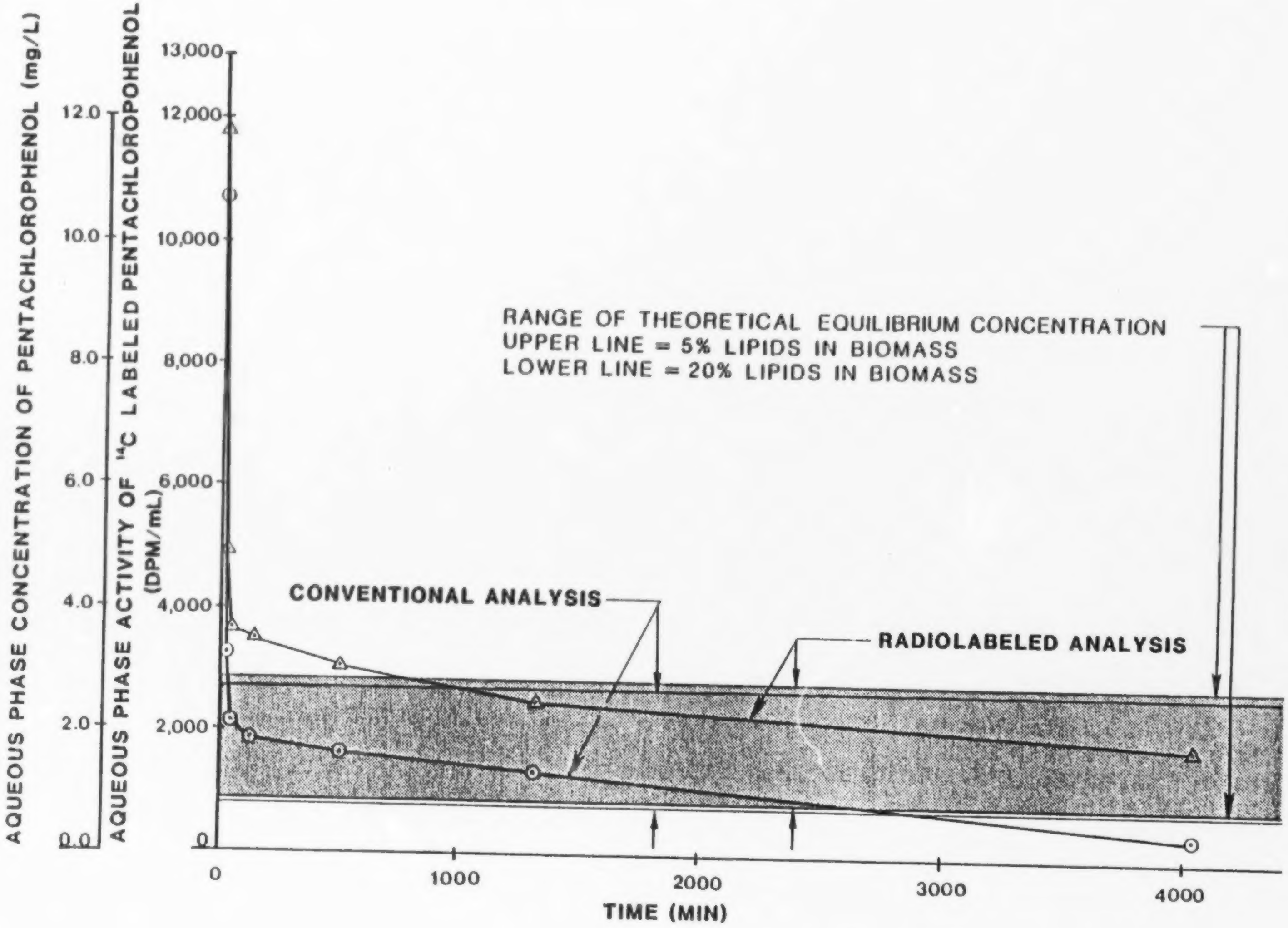

FIG. 32. SORPTION CURVE, PENTACHLOROPHENOL RUN SP-9: LYOPHILIZED BIOMASS

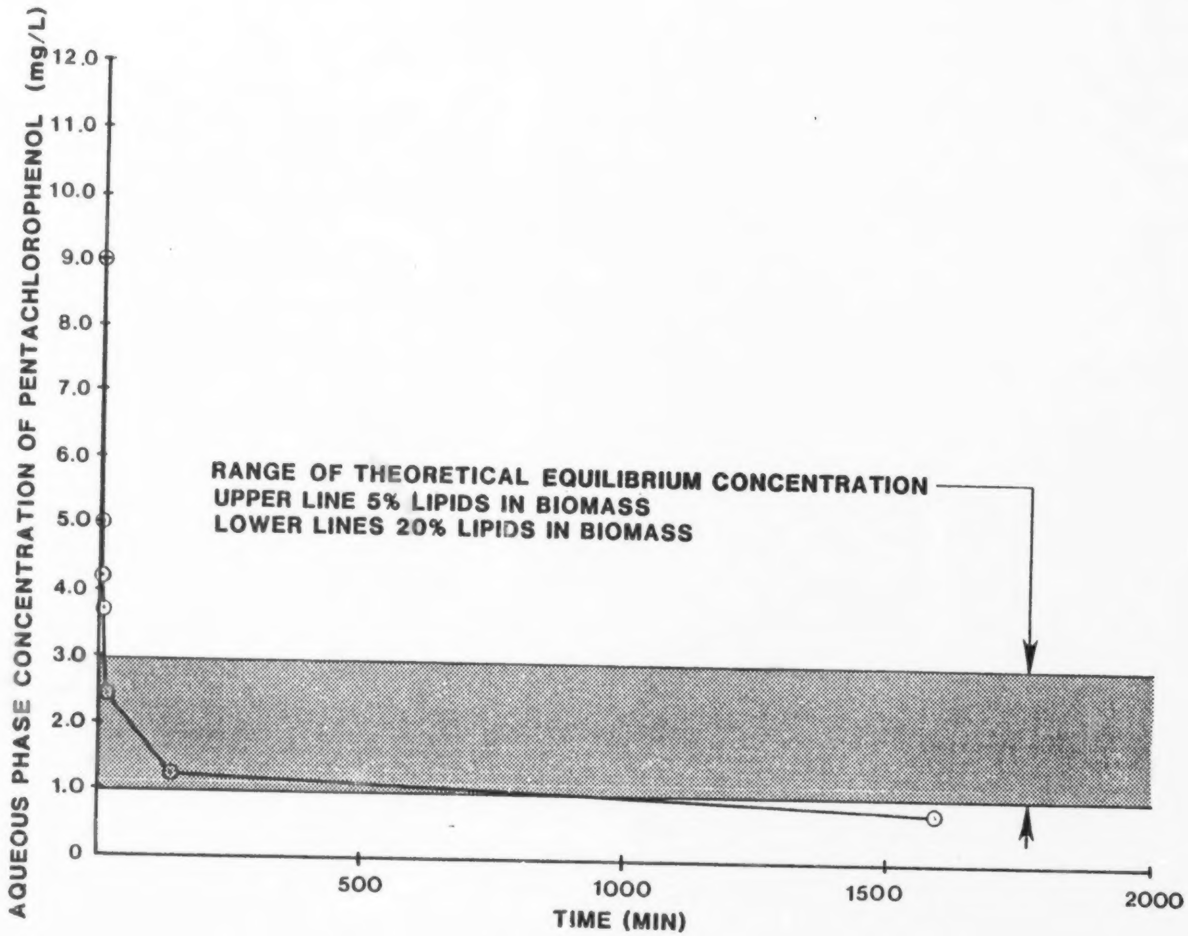

FIG. 33. SORPTION CURVE, PENTACHLOROPHENOL RUN SP-11: LYOPHILIZED BIOMASS

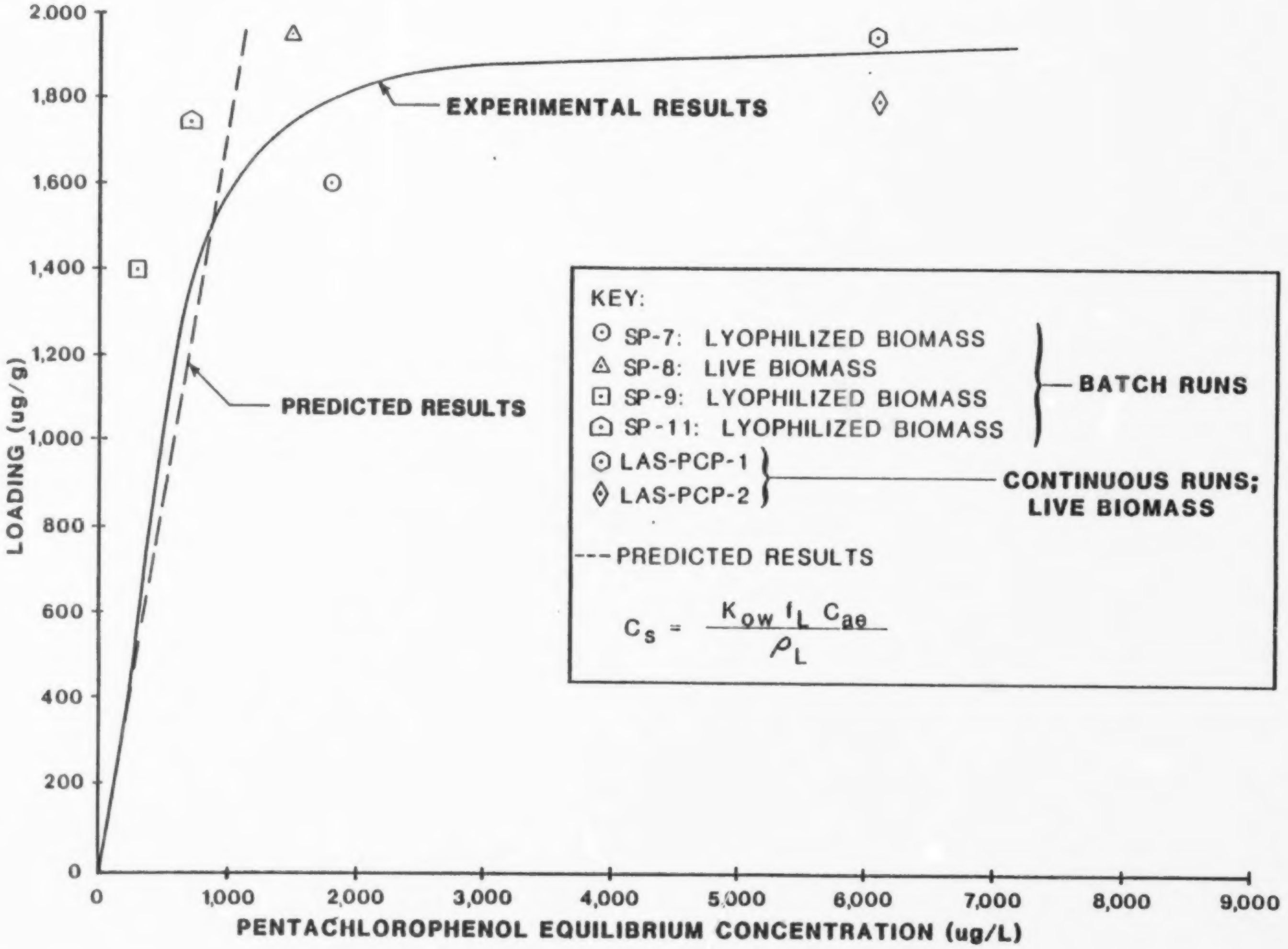

FIG. 34. LOADING VS. EQUILIBRIUM CONCENTRATION, PENTACHLOROPHENOL BATCH AND CONTINUOUS EXPERIMENTS

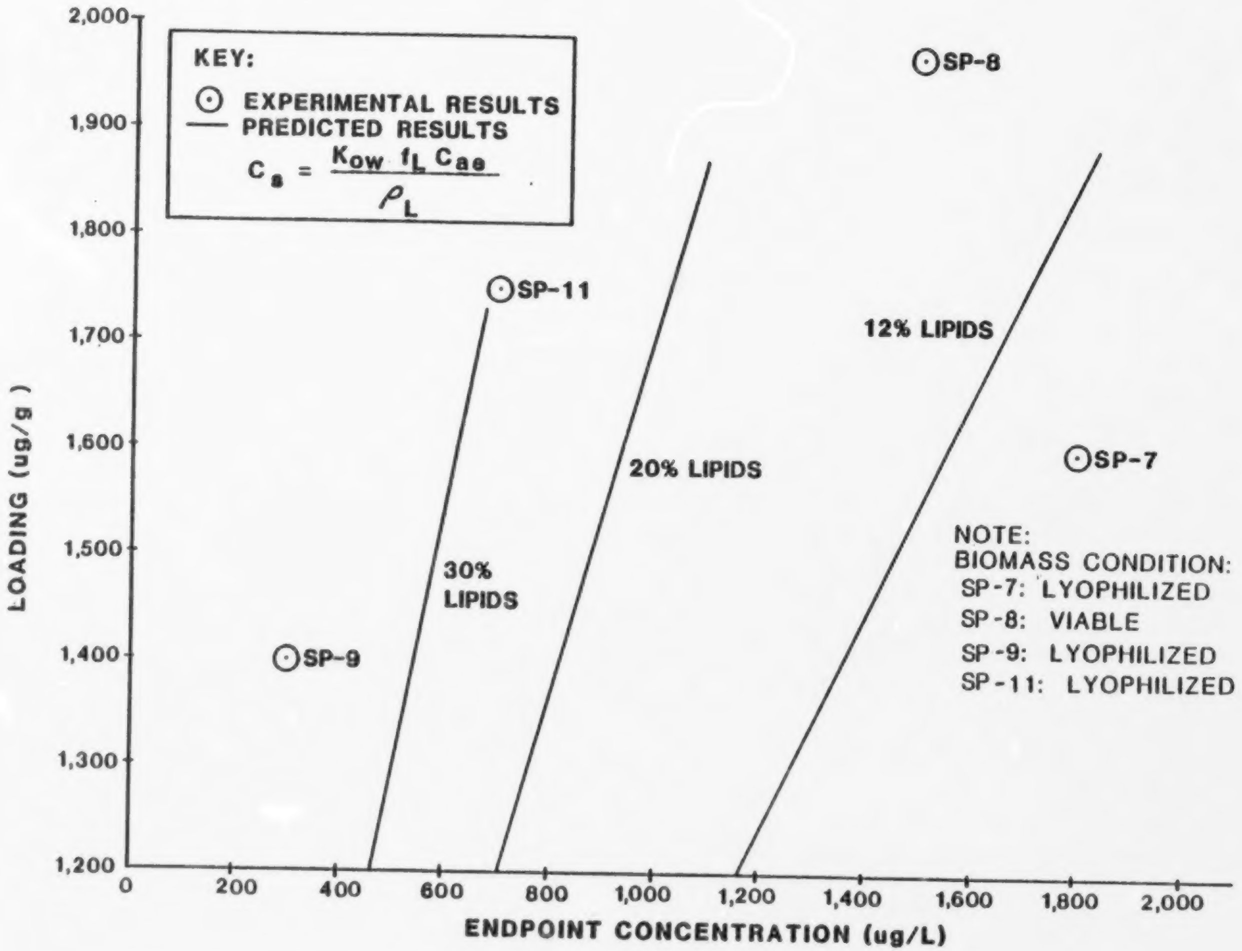

FIG. 35. LOADING VERSUS ENDPOINT CONCENTRATION, PENTACHLOROPHENOL

TABLE 44. PENTACHLOROPHENOL SORPTION RESULTS

Parameter	Run SP-7[a]	Run SP-8	Run SP-9[a]	Run SP-11
Reactor starting[b] concentration (mg/L)	6.1	6.5	10.7	9.0
Endpoint concentration (mg/L)	0.8	1.5	0.3	0.7
Predicted endpoint concentration[c] (mg/L)	0.91	1.22	0.78	0.99
Loading[d] (mg PCP/g dry biomass)	1.6	2.0	1.4	1.7
Predicted loading[c] (mg PCP/g dry biomass)	1.6	2.1	1.3	1.7
Material balance closure (%)[e]	f	f	f	f
Reactor biomass, dry basis (g)	30.3	23.1	66.4	46.4
Total aqueous volume in reactor (L)	9.1	9.1	8.9	9.8
Biomass condition	Lyophilized	Viable	Lyophilized	Lyophilized

[a] Corresponding radiolabeled analysis found in Table 48.

[b] See Eqn. 53.

[c] Prediction based on 20% lipid content, using K_{ow} prediction equations.

[d] See Eqn. 52.

[e] Material balance closure = $\frac{\text{compound recovered from solid phase}}{\text{compound lost from aqueous phase}}$ X 100.

[f] Balance not closed. Insufficient data.

The sorption results compared to predictions using Eqns. 49 and 50 are summarized for all three compounds in Tables 45, 46, and 47. Table 45 shows the measured endpoint concentration, the concentration predicted using Eqn. 49 with $f_L = 0.2$ and $\rho_L = 900$ g/L. A relative percent deviation between the measured values and the predicted values is also calculated. Phenol predictions were quite good but they would be expected to deviate only slightly since the overall change in concentration in the experiment is small. Relative percent deviations for 1,4-dichlorobenzene ranged from +37.6 to -29.6%. This is acceptable given the scope of the study and the limited nature of the investigation. Except for experiment SP-9, the relative percent deviations were in the range of +18.6 to -13.8% for PCP. All PCP predictions were of the same order as the measured values.

TABLE 45. COMPARISON OF MEASURED AND PREDICTED SORPTION ENDPOINT CONCENTRATIONS FOR BATCH EXPERIMENTS

Compound	Experiment	Measured concentration (mg/L)	Predicted concentration[a] (mg/L)	Percent[b] deviation
Phenol	SP-3	73.0	74.2	-1.6
	SP-4	73.3	74.6	-1.8
	SP-5	71.8	72.8	-1.4
	SP-6	76.8	75.7	1.4
1,4-Dichlorobenzene	SP-1	26.6	17.8	33.0
	SP-2	54.2	33.8	37.6
	SP-10	13.9	14.3	2.9
	SP-11	2.7	3.5	-29.6
Pentachlorophenol[c]	SP-7	0.8	0.91	-13.8
	SP-8	1.5	1.22	18.7
	SP-9	0.3	0.78	-160
	SP-11	0.7	0.99	-41.4

[a] Predicted based on Eqn. 49 where $f_L = 0.2$ and $\rho_L = 900$ g/L.

[b] Percent deviation = (measured value - predicted value) ÷ measured value X 100.

[c] Pentachlorophenol partially ionized at pH = 7, $K_{ow} = 7700$.

Another test of Eqn. 49 can be developed if percent removals are calculated with the starting concentrations and the measured and predicted endpoint concentrations. This comparison is presented in Table 46. Except for runs SP-1 and SP-2, the initial two experiments, the difference in the measured and predicted removals ranges from +6.3 to -43%. The first two experiments were shakedown experiments and more variation in the results are expected. This is an exceptional result considering that the octanol-water partition coefficients of the compounds studied range from 30 to 7700. Batch percent removal calculations based on Eqn. 49 appear to be reliable for the endpoint concentrations studied in these tests.

TABLE 46. COMPARISON OF SORPTION PERCENT REMOVAL MEASURED AND PREDICTED USING EQN. 49

Compound	Experiment	Measured removal (%)	Predicted removal[a] (%)	Difference[b] (%)
Phenol	SP-3	4.6	3.0	1.6
	SP-4	5.8	4.1	1.7
	SP-5	3.9	2.5	1.4
	SP-6	1.3	2.7	-1.4
1,4-Dichlorobenzene	SP-1	43.0	61.9	-18.9
	SP-2	30.0	56.4	-26.4
	SP-10	72.7	72.0	0.7
	SP-11	78.7	72.4	6.3
Pentachlorophenol[c]	SP-7	86.9	85.1	1.8
	SP-8	76.9	81.2	-4.3
	SP-9	97.2	92.7	4.5
	SP-11	92.2	89.0	3.2

[a] (Initial concentration - endpoint concentration) ÷ initial concentration X 100.

[b] Difference = measured removal - predicted removal.

[c] Pentachlorophenol partially ionized at pH = 7, K_{ow} = 7700.

Equation 50 is tested in Table 47. For all compounds, the predicted loadings are of the same order as the measured loadings. The relative percent deviation varies from +37.5 to -87.8%. Particularly encouraging is the very good agreement seen in the pentachlorophenol experiments.

The maximum deviations of nearly all parameters predicted certainly falls within ±100% of the measured values. For 26 compounds where soil partitioning is predicted from an empirical equation based on the K_{ow} of the compound the mean percent relative deviation between compounds is of the order of ±200%.[3] The precision of empirical equations in use to predict bioconcentration factors of many compounds are said to be within one order of magnitude or ±500% of the true value.[3]

We believe that the proposed equation, upon further testing and verification and further development of supporting analytical methods, offers hope of an improved predictive methodology for biomass sorption in treatment processes.

TABLE 48. RADIOLABELED COMPOUND RESULTS

Parameter	Phenol Run SP-5	Phenol Run SP-6	DCB Run SP-10	DCB Run SP-11	PCP Run SP-7	PCP Run SP-9
Reactor starting concentration (DPM/mL)	19,751	11,729	6,719.3	8,467.0	12,879.5	11,831.8
Endpoint concentration (DPM/mL)	13,192	8,376	953.5	1,726.2	2,805.5	1,870.8
Predicted endpoint concentration[a] (DPM/mL)	19,254	11,418	1,891	2,363	1,922.9	859.4
Loading (DPM/mg dry biomass)	416.0	264.9	1,370.1	1,402.9	2,172.8	1,032.1
Predicted loading[a] (DPM/mg dry biomass)	100.7	62.2	1,028.1	1,271.1	3,315.6	1,470.8
Material balance closure (%)[b]	84.9	45.9	111	99	79.6	77.2
Biomass condition	Lyophilized	Lyophilized[c]	Lyophilized	Lyophilized	Lyophilized	Lyophilized

[a] Prediction based on 20% lipid content using K_{OW} prediction equations.

[b] Material balance closure = $\frac{\text{compound recovered from solid phase}}{\text{compound lost from aqueous phase}} \times 100.$

[c] Cooled to 4°C.

TABLE 47. COMPARISON OF MEASURED AND PREDICTED LOADING FOR SORPTION BATCH EXPERIMENTS

Compound	Experiment	Measured loading (mg/g)	Predicted loading[a] (mg/g)	Percent deviation
Phenol	SP-3	0.8	0.5	37.5
	SP-4	0.7	0.5	28.6
	SP-5	0.8	0.5	37.5
	SP-6	0.3	0.5	-66.7
1,4-Dichlorobenzene	SP-1	6.7	9.7	-44.8
	SP-2	9.8	18.4	-87.8
	SP-10	7.9	7.8	1.3
	SP-11	2.7	1.9	29.6
Pentachlorophenol[b]	SP-7	1.6	1.6	1.9
	SP-8	2.0	2.1	-4.0
	SP-9	1.4	1.3	5.0
	SP-11	1.7	1.7	0.6

[a] Based on Eqn. 50.

[b] Pentachlorophenol partially ionized at pH = 7, K_{ow} = 7700.

Radiolabeled Compound Results

Endpoint concentrations, loadings, and sorption rate constant data for the six radiolabeled runs are presented in Figs. 22, 23, 27, 28, 30, 32 and Tables 48 and 49. Where comparison is possible, the results obtained using radiolabeled compounds support those obtained using conventional compounds.

TABLE 49. MINIMUM SORPTION RATE VALUES, RADIOLABELED ANALYSIS

Compound	Run	Sorption kinetic rate constant, K_a^{sp} (hr^{-1}) 1st step	2nd step
Phenol	SP-5	5.5	
Phenol	SP-6	5.3	
1,4-Dichlorobenzene	SP-10	22.1	
1,4-Dichlorobenzene	SP-11	10.7	
Pentachlorophenol	SP-7	0.8	
Pentachlorophenol	SP-9	1.3	0.04

Radiolabeled results from the phenol experiments generally have the same shaped concentration-time profile as conventionally measured phenol. However, quantitative comparison leads to the conclusion that there is some experimental or analytical anomaly in either the conventional or radiolabeled data because the percent of the compound loading is somewhat different in each case. The radiolabeled data are probably more tenuous because of the limited experience available in measuring ^{14}C substrate in biological solids using solids digestion techniques. Time and budget limitations prevented further study of this discrepancy and the cause remains unknown. The conventional analysis is considered to be more credible data because of the broader experience with this data and because of the QA/QC results reported in Appendix B. It is again noted that the initial concentration is very important in making these calculations and in every case, the initial concentration is a calculated value. In any case, the phenol radiolabeled analysis is provided in this report to corroborate the general shape of the conventional profile curve.

Material Balance Closure

Material balance calculations were performed for each conventional and radiolabeled experiment for which sufficient data were available. These calculations, the results of which are noted in Tables 41, 43, 44, and 45, serve as a general guide to the reliability of the data from each run. The formulas used in the material balance are detailed in Appendix E. The material balance closure is defined as the organic analytically recovered from the solid phase divided by the organic lost from the aqueous phase.

QA/QC Results

Ninety-five percent of the QA related data, which includes instrumental precision, method precision, accuracy, and audit sample results, met the project QA objectives. QA/QC objectives are detailed in Appendix D entitled Quality Assurance Summary.

SECTION 9

DEVELOPMENT OF A BIO-OXIDATION PREDICTIVE FATE METHOD

SUMMARY

The contribution of bio-oxidation to predictive fate assessment of organic pollutants in industrial waste treatment was determined for batch and continuous flow laboratory scale bioreactors. Bio-oxidation of specific pollutants was measured in activated sludge samples under batch assay conditions. Mineralization (oxidation to CO_2) of ^{14}C-labeled pollutants was chosen as a primary, unambiguous measurement of bio-oxidation.

Qualitative and quantitative measurements of specific degradative bacterial populations comprising the activated sludge were performed. These measurements and associated enzymatic activities were used to evaluate the potential for describing bio-oxidation kinetics as a function of the biological community. In addition, the potential for predicting qualitative and quantitative pollutant fate relative to sludge composition and activity was investigated.

Development of the batch kinetic bio-oxidation assay was performed by varying experimental parameters such as sludge cell density, substrate concentration, sludge acclimation and incubation time. Surveys of enzyme activity were conducted to select candidate enzyme activities useful in evaluation of bio-oxidation performance. Direct microscopic counting procedures and viable colony forming units on sole carbon source plating media were used to measure microbial population densities.

Bio-oxidation kinetics were measured for phenol, toluene, and 2,4-dichlorophenol in sludge obtained from fill-and-draw reactors; while phenol, toluene, and aniline bio-oxidation kinetics were examined in samples from continuous flow reactors. Bio-oxidation kinetics appeared to be first order rate processes relative to pollutant concentration and concentration of degradative bacteria. The quantitative composition of degradative bacteria in the sludge directly influenced rates of bio-oxidation. Degradative bacterial populations were highly responsive to acclimation to the individual substrates. In addition, under fill-and-draw culture condition feed stock influenced acclimation time and rates of bio-oxidation. This variability was overcome by inclusion of an in-line second stage fill-and-draw reactor using a depleted feed stock from the first stage effluent. Problems in enumeration of degradative populations and maintenance in the sludge were associated with pollutant toxicity and quality as a growth supporting

substrate. Enzymatic activity associated with bio-oxidation of aromatic pollutants were demonstrated in the sludge, but at low levels. This effect was attributed to the relative low density of degradative bacteria relative to the total sludge bacterial community.

Comparatively, first order rate constants (K_1^{bm}) for mineralization indicated that a relative ranking of bio-oxidation rates could be achieved, i.e., aniline>phenol>toluene with mean K_1^{bm} values of 4.3 X 10^{-2}, 1.6 X 10^{-2}, and 4.5 X 10^{-3} hr^{-1}, respectively. However, this relationship changed when the concentration of specific degradative bacteria was used to develop a second order kinetic rate constant (K_2^{bm}). While aniline remained the easiest pollutant to bio-oxidize (K_2^{bm} = 2.6 X 10^{-9}), toluene exceeds phenol in bio-oxidation potential; K_2^{bm} values 1.9 X 10^{-9} and 2.1 X 10^{-10}, mL $org^{-1}hr^{-1}$, respectively. These bio-oxidation kinetics were found to be similar to those reported for pure culture studies. The results of the investigation indicate that the potential exists for predicting the bio-oxidative capacity of activated sludge as well as enhancing its bio-oxidative capacity.

Specific conclusions and recommendations follow.

Specific degradative bacterial populations in activated sludge comprise less than 0.1% of the total bacterial population.

Maximum degradative bacterial population densities and rate of pollutant bio-oxidation are achieved after prolonged acclimation of 2 to 3 weeks for relatively easily bio-oxidized pollutants such as phenol and toluene.

Specific catabolic enzyme activities are detectable in activated sludge but are in low concentration as a result of low degradative cell densities. Larger biomass samples are needed to allow quantitation.

The biochemical complexity of substrate mineralization results in first order mineralization kinetic rate constants that are an order of magnitude slower than substrate disappearance rate constants. However, mineralization represents an unambiguous predictor of pollutant destruction.

Fill-and-draw and continuous flow reactors appear to develop similar degradative populations that are reflected in similar bio-oxidation responses under mineralization assay conditions.

Bio-oxidation kinetics can be described as first order rate processes for each substrate concentration and the concentration of degradative bacterial cells.

Pollutant toxicity and feed stock repression result in decreased rates of bio-oxidation by suppressing degradative cell densities and inhibiting biochemical pathways for mineralization.

Toxic and/or poor growth-supporting pollutant substrates will require modification in bio-oxidation assay procedures and estimation of degradative populations to achieve accurate predictions of bio-oxidation kinetic parameters.

Bio-oxidation kinetic responses observed lie within realistic limits reported for pure culture studies. The results indicate that on a per cell basis, degradative populations are efficiently oxidizing the pollutant and that the limits of bio-oxidation in activated sludge are controlled by the density of degradative bacteria.

Under the experimental conditions employed, the bio-oxidation mineralization rate (first order) of aniline is faster than toluene, which in turn is faster than phenol. Second order rate constants result in a different conclusion.

Consortium or co-oxidative metabolism of substrates such as 2,4-dichlorophenol results in relatively rapid bio-oxidation that requires greater definition of parameters controlling kinetic responses.

There is a major need to delineate factors controlling maximum degradative population densities in activated sludge. Such factors once identified may be manipulated to enhance the overall bio-oxidative capacity for specific pollutants. (These may include feedstock, protozoan grazing, toxicity, etc.)

The detection and enumeration of specific degradative populations needs to be refined to achieve better resolution of the kinetic parameters of bio-oxidation. This is especially true for the more recalcitrant and/or toxic pollutants. New available genetic probing techniques can be employed to quantitatively and qualitatively characterize degradative genes within the mixed sludge community. Such techniques may be invaluable in assessing mixed culture or consortium bio-oxidation where no single degradative population can utilize a pollutant as a growth substrate or directly promote mineralization.

Methodology aimed at development of substrate disappearance rate measurement and kinetic order determination needs to refined. It is necessary that these methods be capable of accurately determining disappearance rates in large-scale continuous facilities.

The role of sludge recycling in maintaining a degradative gene pool, slow growing degradative bacteria within the continuous flow bio-reactor, and cycling of substrate and intermediate products needs to be evaluated for assessing enhanced reactor performance and stability.

Variables resulting in differences in kinetic rate constants under fill-and-draw and continuous flow conditions need to be identified to refine the mineralization assay as an accurate predictor of bio-oxidation kinetics.

Evaluation of bio-oxidation kinetics at wide ranges (high and low) of substrate concentration should be evaluated to increase predictive accuracy and to determine the order of the rate equation. This may include scale-down of continuous bio-reactors for mineralization assessment.

Scale-up of enzymatic assay methods is needed to achieve the desired sensitivity to make enzyme assays a useful predictor of bio-oxidation potential.

The comparative qualitative similarity of degradative bacteria in continuous and fill-and-draw reactors, during and after acclimation, should be addressed to determine the efficiency of batch culture prediction. This analysis could be conducted at the genetic, taxonomic, or enzymatic level.

The spectrum of pollutant substrates subject to investigation should be broadened to more recalcitrant substrates. The choice of substrates should be made relative to the available information on biochemical and genetic routes of bio-oxidation in order to achieve the greatest information in bio-oxidation studies.

INTRODUCTION

The goal of biological waste treatment is the oxidation of organic matter to carbon dioxide, water, and inorganic salts. In the context of both industrial and domestic wastes, this goal serves the same intent, to prevent perturbations of natural ecosystems and to reduce or eliminate environmental health risks associated with the release of toxic pollutants. In biological waste treatment systems, the organic constituents of the waste stream serve as both carbon and energy sources for a complex microbial community that is functionally responsible for purification of the waste. Consequently, quantitatively and qualitatively, this community is controlled by the nature of the waste and engineering designs and operation of the waste treatment facility. To achieve maximum biological conversion of organics in the waste stream, in a realistic time frame, high density communities are maintained as a trade-off between conversion of the organics to CO_2 and production of disposable microbial biomass. For most domestic and many industrial wastes, this trade-off is acceptable and efficient. However, for waste streams containing toxic and/or biologically recalcitrant pollutants, highly efficient removal of the bulk of organics in the waste may not provide acceptable reduction of pollutants or of the risk of environmental exposure.

The fate of organic pollutants in biological wastewater treatment is determined by the interaction of physical, chemical, and biological processes that determine the eventual stripping or volatilization, sorption and bio-oxidation (biodegradation) composite fate of the pollutant. In order to accurately assess the efficiency of wastewater treatment designs and to predict the release of potentially hazardous pollutants from the system, it is necessary to quantitatively describe these interactive processes determining the eventual fate of the pollutant. Since treatment systems have a definite and controllable residence time that a mass of pollutant is subjected to biological treatment, it is necessary to kinetically describe the rates at which these competing processes proceed in relation to the eventual destruction of the pollutants.

The rates at which the competing processes occur may limit the rate of bio-oxidation. However, in addition to potential rate limiting physical/chemical processes in activated sludge, a number of additional factors influence both the rate and extent of pollutant bio-oxidation.

A number of chemical factors, both structural properties of the chemical as well as environmental factors, influence the biological recalcitrance (resistance to biodegradation) of organic pollutants (Table 50). Structural properties of the organic pollutant tend to determine the entry of the pollutant into the microbial cell and induction of enzymes responsible for catabolism of the pollutants. Environmental factors exert their primary influence by regulating or defining the biochemical conditions in which synthesized catabolic enzymes are physiologically active. As indicated in Table 51, biological factors interacting with chemical factors further modulate the biodegradation of organic pollutants and the anticipated or predictive performance of biological waste treatment systems. Many of these factors relate to the enzymology of biodegradative capacity. Biodegradative enzymes are not distributed throughout all bacterial species and many of the enzymes have a narrow range of specificity. The specificity of degradative enzymes increases throughout highly regulated biochemical pathways that ultimately produce simple metabolites that can be used to generate cellular energy and carbon. Many of the biochemical pathways may be exclusively encoded in extrachromosomal plasmid DNA. The plasmids themselves are not uniformly distributed among a given species and may be lost from the bacteria with no effect on cell survival. At the population level, individual species will compete for all available nutrients and some species will be more adapted for fixed film habitats rather than suspended growth. In addition, grazing by protozoan populations, parasitic bacterial infections, and bacterial viruses will tend to influence maximum attained densities of individual species within the wastewater community.

While chemical and biological factors influence the attainable bio-oxidation of an organic pollutant, the ability to evaluate and predict the extent and kinetics of bio-oxidation is further affected by methodological and experimental factors (Table 52). These factors include the method used to determine bio-oxidation of the substrate and the applicability of lab-scale protocols to full-scale systems. In addition, feedstocks used for laboratory cultivation and analysis greatly influence extrapolation to full-scale treatment processes.

In an attempt to define and predict the bio-oxidative capacity of activated sludge treatment, this investigation was conducted to evaluate the use of degradative bacterial cell densities and enzyme activities in predicting the bio-oxidation of organic pollutants in biological treatment processes.

TABLE 50. CHEMICAL FACTORS INFLUENCING BIODEGRADABILITY OF ORGANIC POLLUTANTS

	Consequences
Structural properties	
Molecular weight or size	Limited active transport
Polymeric nature	Extracellular metabolism required
Aromaticity	O_2 requiring enzymes
Halogen substitution	Lack of dehalogenating enzymes
Solubility	Competitive partitioning
Toxicity	Enzyme inhibition, cell damage
Xenobiotic origin	Evolution of new degradative pathways
Environmental factors	
Dissolved oxygen	O_2 sensitive and O_2 requiring enzymes
Temperature	Mesophilic temperature optimum
pH	Narrow pH optimum
Dissolved carbon	Organic/pollutant complexes; concentration dependent growth
Particulates, surfaces	Competition for substrate
Light	Photochemical enhancement
Nutrient and trace elements	Limitation on growth and enzyme synthesis

TABLE 51. BIOLOGICAL FACTORS INFLUENCING THE BIODEGRADABILITY OF ORGANIC POLLUTANTS

Biological factors	Consequences
Enzyme ubiquity	Low frequency of degradative species
Enzyme specificity	Analogous substrates not metabolized; re-acclimation
Plasmid encoded enzymes	Low frequency of degradative species; instability
Enzyme regulation	Repression of catabolic enzyme synthesis; required acclimation or induction
Competition	Extinction or low density populations
Habitat selection	Lack of establishment of degradative populations
Population regulation	Low population density of degradative organisms

TABLE 52. FACTORS INFLUENCING THE ANALYSIS OF THE KINETICS AND EXTENT OF BIODEGRADATION

Experimental factors	Problems encountered
Analytical method	
Substrate disappearance	Competing abiotic processes
Biotransformation	Complex analyses
Mineralization	Incomplete biochemical pathways
Scale up/down	Comparability among reactor designs
Feedstock complexity	
Chemically/biologically defined	Poor simulation and predictability
Complex wastewaters	Difficult interpretation

Lewis, et al.[64], recently summarized the kinetic relationships between microbial transformation rates and test chemical concentrations. As indicated by Table 53, multiphasic kinetic rate coefficients are predicted for a range of substrate concentrations, while transformation rate coefficients for substrate removal appear strictly first order at trace chemical levels and appear zero order at elevated chemical concentrations. As indicated by this summary, at substrate concentrations likely to be encountered for individual pollutants in wastewater treatment, microbial biotransformation kinetics are predicted to be multiphasic (mixed reactor order) relative to substrate but first order relative to degradative enzyme (cell) concentration.

Although numerous studies have been performed to evaluate microbial growth kinetics and systems performance characteristics (such as BOD and COD removal) in wastewater containing certain hazardous pollutants, there is little information about the microbial kinetics of oxidation to CO_2 of specific pollutants, disappearance kinetics for specific compounds, and the kinetic relationships to stripping or sorption in the waste treatment process.

Goals and Objectives

The goal of this investigation was to develop fundamental information on bio-oxidation kinetics to predict the fate of organic pollutants in industrial waste treatment processes. This investigation was undertaken in collaboration with scientists and engineers at IT Corporation in order to develop an integrated predictive fate assessment of specific model pollutants.

TABLE 53. SUMMARY OF MICROBIAL TRANSFORMATION RATE INFORMATION BASED ON MICHAELIS-MENTEN KINETICS[a]

Pollutant concentration	Kinetics of microbial transformation
$>10^{-2}$ M	Microbial transformation rates are: ● First-order to enzyme concentration ● Zero-order to pollutant concentration ● Frequently subject to toxic effect of pollutant ● Frequently subject to population adaptation Applicable rate coefficients are:[b] ● k_0
$<10^{-2}$ M $>10^{-7}$ M	Microbial transformation rates are: ● First-order to enzyme concentration ● Multiphasic (series of first-mixed and zero-order) to pollutant concentration ● Sometimes subject to toxic effect of pollutant ● Sometimes subject to population adaptation Applicable rate coefficients are:[b] ● k_1, k_2, k_A
$<10^{-7}$ M	Microbial transformation rates are: ● First-order to enzyme concentration ● First-order to pollutant concentration ● Rarely subject to toxic effect of pollutant ● Rarely subject to population adaptation Applicable rate coefficients are:[b] ● k_1, k_2, k_A

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[a] From Ref. 64.

[b] Transformation rate coefficients are based on rates of loss of parent compound. K_1 (hr^{-1}) and K_2 (L $cell^{-1}$ hr^{-1}) are first and second order rate constants as previously described in text. K_0 (mg L^{-1} hr^{-1}) is a first order rate constants zero order in substrate and first order in biomass; while K_a (L m^{-2} hr^{-1}) is first order per ratio of periphyton-colonized surface area to volume.

The primary objectives of this research project included:

- Determine the potential for predicting the bio-oxidative fate (mineralization) of pollutants as a function of bacterial species responsible for oxidation of a single pollutant chemical in activated sludge
- Assess the utility of enzyme activity measurements in predicting the bio-oxidation potential of sludge biomass

- Develop quantitative bio-oxidation protocols to predict pollutant fates in wastewater treatment and improve the bio-oxidation capacity of activated sludge

For this study, a group of aromatic substrates was chosen. These substrates exhibit moderate toxicity and recalcitrance and were chosen to develop methods and test experimental hypotheses that were attainable within the time frame of this investigation.

BIOCHEMISTRY OF THE METABOLISM OF AROMATIC COMPOUNDS

Organic compounds are catabolized by microorganisms by three general processes: (1) Fermentation takes place in the absence of oxygen and requires no external electron acceptor; although CO_2 is often formed, incompletely oxidized products are principal products; (2) Anaerobic respiration is an anaerobic process that utilizes inorganic electron acceptors such as NO_3^-, SO_4^{--}, and CO_2; organic compounds, including aromatic molecules such as phenol may be converted to organic acids that enter the metabolic pool; some CO_2 may be evolved; and (3) Aerobic respiration in which O_2 serves as the terminal electron acceptor; the substrates are converted to CO_2, water, cell mass, and incompletely oxidized products. In addition to the three basic processes, communities of microorganisms have available various types of interactive processes that lead to the complete degradation of organic compounds to CO_2, or in some instances to the release of CH_4 under anaerobic conditions. In this investigation, aerobic respiration of aromatic pollutants was examined as the primary route of bio-oxidation in activated sludge biological treatment.

The degradation of aromatic compounds by aerobic bacteria involves enzymatically catalyzed reactions that first cleave the benzene nucleus and subsequently form compounds compatible with the tricarboxylic acid (TCA) cycle where they serve as substrates for growth and energy production via oxidative phosphorylation.[65,66] Before the benzene nucleus can be cleaved, it generally must have at least two hydroxyl groups that are either ortho or para to one another. If this requirement is not met by the substrate molecule, one or both hydroxyls must be inserted in the proper position. Enzymes that catalyze placement of hydroxyl groups on a benzene nucleus are called monooxygenases (or sometimes hydroxylases). Since only one atom of oxygen is inserted in the ring, monooxygenases require a second electron donor to reduce the second oxygen atom from O_2 to water. In most instances a reduced coenzyme, NADH or NADPH, donates electrons to a flavoprotein coenzyme that interacts with the monooxygenase.

Dioxygenases are generally involved in the insertion of adjacent hydroxyl groups from the direct incorporation of two atoms of molecular oxygen onto the aromatic ring. The cleavage of the ring is accomplished by dioxygenases that insert both atoms of molecular oxygen into the benzene nucleus, forming a dicarboxylic acid or a semialdehyde with a carboxylic acid group at one terminus and an aldehyde at the other.

The metabolic pathways for the conversion of aromatic compounds to TCA cycle intermediates have been extensively studied in fungi and bacteria. Much of the information about the metabolism of the compounds used in this study--phenol, toluene, and aniline--comes from investigations with species of the genus Pseudomonas, particularly P. putida. The details of the metabolism of toluene, xylene, and their alcohol, aldehyde, and carboxylic acid derivatives are well known.[67-82] Aniline has been shown to be readily degraded by various bacteria, but this compound does not appear to have been used extensively in studies with the pseudomonads.[72,82-84] On the basis of some experiments in which oxygen uptake and product formation were measured, it appears that catechol is an intermediate in the utilization of aniline, as it is for phenol and toluene. The following discussion of the degradation of these three substrates is based on information derived from experiments with the pseudomonads.

Figure 36 shows the first steps in the utilization of aniline and phenol. Although other reactions are possible, dioxygenase attack on the C-N bond of aniline releasing ammonia and forming catechol is probable based on scant information in the literature. Phenol is acted on by an oxygenase to form catechol. Catechol is cleaved by one of two dioxygenases, thus opening the ring. These reactions are discussed below.

Figure 37 shows the sequence of reactions resulting in the conversion of toluene to catechol. The first step is catalyzed by a monooxygenase converting toluene to benzyl alcohol. The next two steps are catalyzed by dehydrogenases: benzyl alcohol is oxidized first to benzaldehyde and then to benzoate. The enzyme benzoate oxygenase catalyzes a dicarboxylation and a hydroxylation of benzoate to produce catechol. Alternatively, toluene can be directly oxidized to methyl catechol.

Two pathways exist for the cleavage of the benzene nucleus of catechol: an ortho-pathway that opens the ring between carbon atoms 1 and 2, and a meta-pathway that opens the ring between carbons 2 and 3. The genes that control the synthesis of the enzymes for these two pathways are carried by two separate genetic elements in P. putida. The genes for the ortho-pathway are on the bacterial chromosome and are induced most specifically by benzoate. The genes for the meta-pathway reside on a plasmid, an extrachromosomal genetic element. Plasmids are found in nearly all species of bacteria and carry genes for dispensable functions; chromosomal genes are regarded as controlling enzymes needed for central metabolism. In the pseudomonads, a number of plasmid types have been discovered which contain the genes for the degradation of a variety of organic compounds such as camphor, salicylate, naphthalene, and octane. The TOL plasmid of P. putida encodes all of the enzymes for the oxidation of a variety of substituted benzenes providing terminal substrates used in energy production and cellular biosynthesis.

Depending on the strain of bacteria involved, catechol is metabolized by way of either the ortho- or the meta-pathways. The ortho-pathway has β-ketoadipate as an intermediate, but the meta-pathway does not. Figure 38 shows the ortho-pathway in which a dioxygenase, catechol 1,2-oxygenase, converts catechol to cis, cis-muconate. A lactonizing enzyme and an isomerase

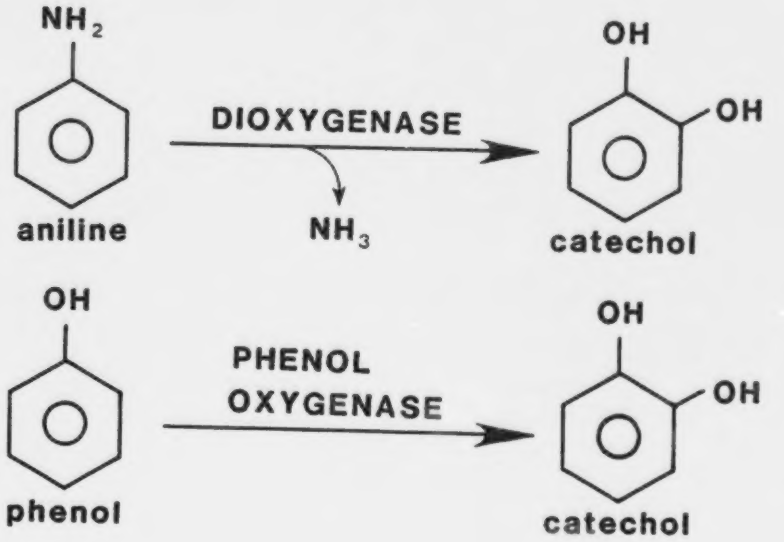
NH_2
aniline
DIOXYGENASE
NH_3
OH
OH
catechol
OH
phenol
PHENOL
OXYGENASE
OH
OH
catechol

FIG. 36. THE CONVERSION OF ANILINE AND PHENOL TO CATECHOL

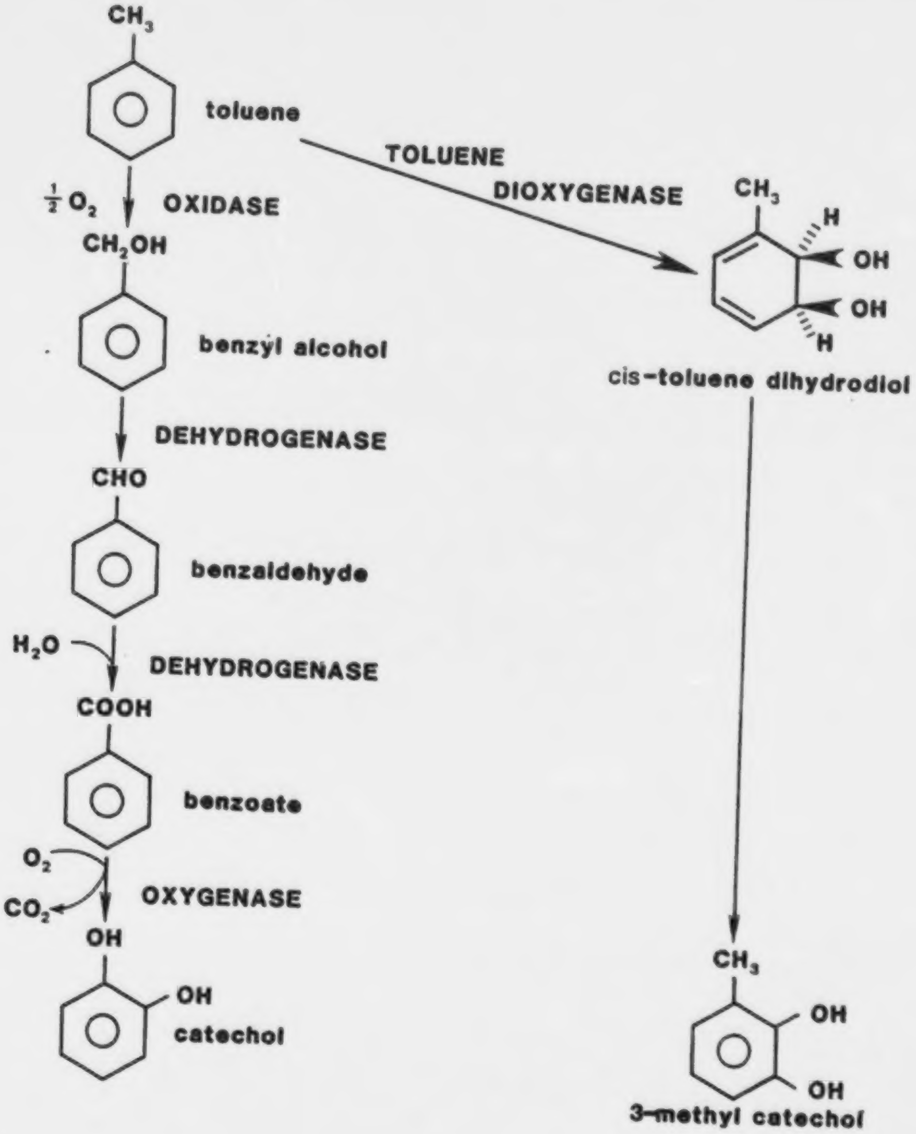
CH$_3$
toluene
TOLUENE
DIOXYGENASE
½ O$_2$
OXIDASE
CH$_2$OH
benzyl alcohol
DEHYDROGENASE
CHO
benzaldehyde
H$_2$O
DEHYDROGENASE
COOH
benzoate
O$_2$
CO$_2$
OXYGENASE
OH
OH
catechol
CH$_3$
H
OH
OH
H
cis-toluene dihydrodiol
CH$_3$
OH
OH
3-methyl catechol

FIG. 37. THE FORMATION OF CATECHOL AND 3-METHYL CATECHOL FROM TOLUENE

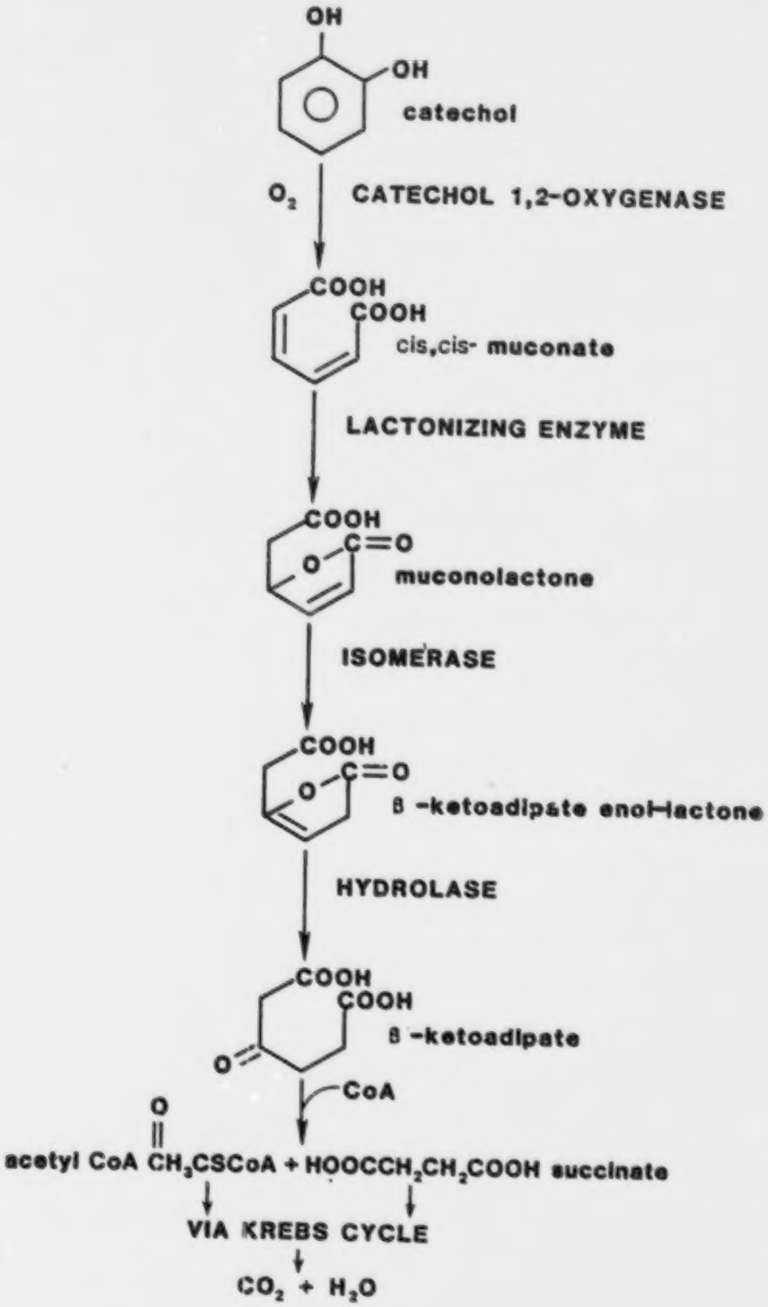
OH
OH
catechol
O_2
CATECHOL 1,2-OXYGENASE
COOH
COOH
cis,cis- muconate
LACTONIZING ENZYME
COOH
C=O
O
muconolactone
ISOMERASE
COOH
C=O
O
β -ketoadipate enol-lactone
HYDROLASE
COOH
COOH
β -ketoadipate
O
CoA
O
acetyl CoA CH_3CSCoA + $HOOCCH_2CH_2COOH$ succinate
VIA KREBS CYCLE
CO_2 + H_2O

FIG. 38. THE ORTHO PATHWAY FOR CATECHOL METABOLISM

cause the formation of β-ketoadipate-enolactone which in turn is acted on by hydrolases to form β-ketoadipate. This final compound is cleaved by enzymes that require coenzyme A to form acetyl-CoA and succinate, two compounds that are metabolized further by the TCA cycle. The initial substrate molecules are converted to CO_2 and water by the complete functioning of this sequence of reactions.

Figure 39 illustrates the meta-pathway for catechol degradation. The first step is catalyzed by a dioxygenase that has a different function than the one in the ortho-pathway. Catechol 2,3-oxygenase adds an atom of oxygen to both the number 2 and number 3 carbons, cleaving the ring. The compound formed by this reaction, 2-hydroxy-muconic-semialdehyde, has two possible fates. A hydrolase may convert it directly to 2-oxopentenoate, or a dehydrogenase may catalyze the formation of the enol form of 4-oxalo-crotonate. After tautomerization to the keto form, this latter compound is decarboxylated to form 2-oxopentenoate. The addition of water by a hydratase forms 4-hydroxy-2-oxovalerate which is in turn split by aldolase to form pyruvate and acetaldehyde. These last two compounds are readily metabolized through the TCA cycle forming CO_2 and water.

Some organisms possess the enzymes of both the ortho- and meta-pathways when the TOL plasmid is present and functional. It should be emphasized that these pathways are representative of the catabolism of aromatic compounds, and that other pathways are used to degrade compounds with different substitutions on the benzene ring.

Several phenomena affect the utilization of nutrient substrates by bacteria; two will be mentioned here but will not be developed further. The first is the matter of entry of the organic molecule into the cell. In the bacteria there are few known examples of the passive diffusion of organic compounds across the cell membrane. Although, relatively few aromatic substrates have been studied in detail, several types of transport mechanisms, some of which require metabolic energy, are known to carry the organic compound across the membrane. All of these processes involve proteins that are integral parts of the membrane and some require additional proteins that are peripheral to the membrane. Such proteins are often subject to genetic induction; that is, a particular molecule must be present that induces the cell to synthesize the protein. The systems are not infallible for various reasons, one of which is mutation in some part of the regulatory process that prevents the induction of the transport protein(s). It is not unusual to find intracellular enzymes for the metabolism of a substrate only to have the membrane transport process non-functional. In such instances, the substrate compound cannot be metabolized by the living organisms.

A second type of phenomenon has been touched on above--the regulation of synthesis of cellular proteins including the enzymes of the catabolic pathways. Particular substrates are necessary to induce the synthesis of the first enzyme in a metabolic series, or sometimes to coordinately induce the synthesis of a set of enzymes that act sequentially in a metabolic pathway. For the conversion of toluene (or xylene) by way of the reactions shown in

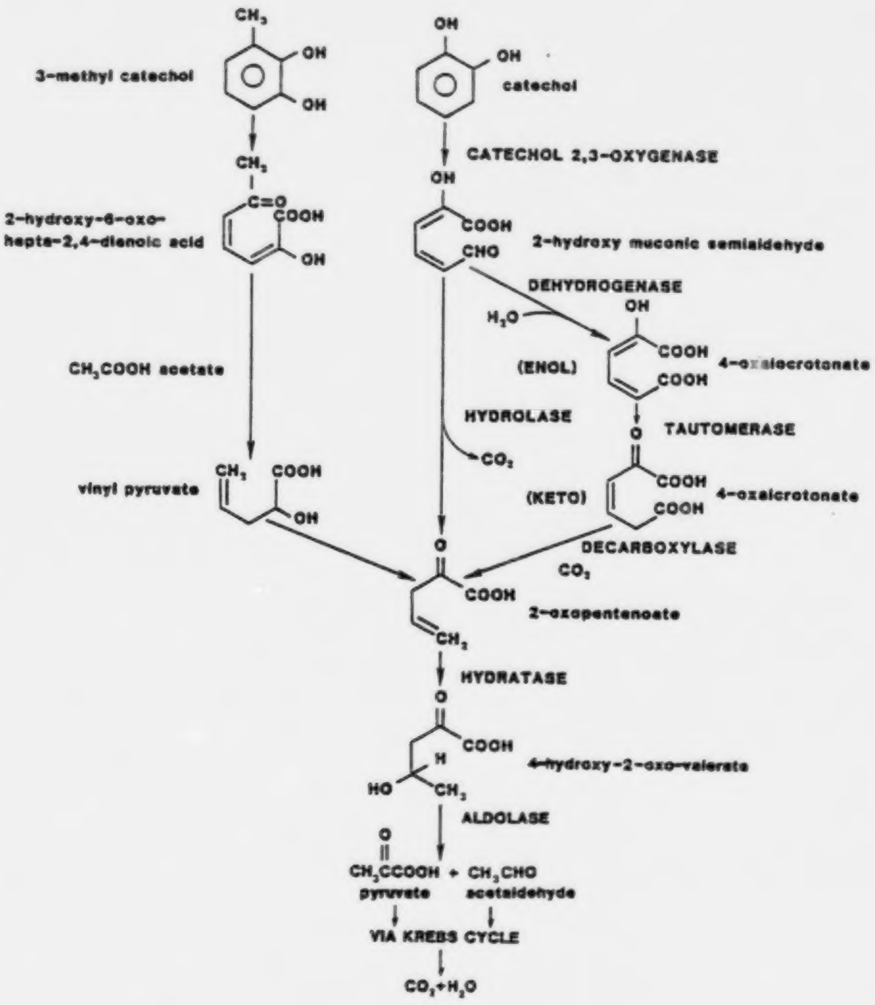
3-methyl catechol
catechol
CATECHOL 2,3-OXYGENASE
2-hydroxy-6-oxo-hepta-2,4-dienoic acid
2-hydroxy muconic semialdehyde
DEHYDROGENASE
H_2O
(ENOL)
4-oxalocrotonate
CH_3COOH acetate
HYDROLASE
CO_2
TAUTOMERASE
vinyl pyruvate
(KETO)
4-oxalcrotonate
DECARBOXYLASE
CO_2
2-oxopentenoate
HYDRATASE
4-hydroxy-2-oxo-valerate
ALDOLASE
CH_3CCOOH + CH_3CHO
pyruvate
acetaldehyde
VIA KREBS CYCLE
CO_2+H_2O

FIG. 39. POSSIBLE META PATHWAYS FOR CATECHOL AND METHYL CATECHOL METABOLISM

Fig. 37 and Fig. 39 (the meta-pathway), a model for regulation has been proposed in which there are two regulatory units controlled by two genes carried on the TOL plasmid. The xylR gene functions with specific categories of inducer molecules, hydrocarbons and alcohols, to control the synthesis of the enzymes that convert toluene to benzoate (Fig. 37) as well as the second set of the enzymes that convert benzoate by way of catechol to pyruvate and acetaldehyde (Figs. 37 and 39). A second regulatory gene, xylS, interacts with its specific inducers (carboxylic acids) to induce only the enzymes in the latter part of the pathway beginning with benzoate oxygenase. An additional regulatory element has been proposed that responds to non-methylated substrates for the induction of the latter part of the pathway. Methylated substrates and alcohols act by way of xylR and xylS genes to induce the last part of the pathway.

METHODS AND PROCEDURES

Experimental Design

Research was conducted in a dual experiment format to test hypotheses and refine experimental methods, and to develop quantitative data on bacterial bio-oxidative performance in "real world" waste treatment. Initial studies were conducted using single substrate, acclimated activated sludge cultures to develop methods used for the measurement of rates of substrate mineralization (oxidation to CO_2). These methods utilized small batch assays and were used to quantify mineralization from two types of biological experiments. Early experiments were conducted using small scale (300 mL) fill-and-draw bio-reactors maintained on a foul condensate (kraft paper pulp) waste effluent. Later experiments were conducted to determine the contribution of bio-oxidation measurements to the overall prediction of pollutant fate in activated sludge treatment. These studies were conducted in 11-L, continuous feed bio-reactors using a heterogeneous acclimated sludge, maintained on a foul condensate feed stock, and challenged by the addition of individual test pollutant substrates.

Chemicals and Reagents

Reagent grade toluene, phenol, and aniline (all Fisher Scientific) and 2,4-dichlorophenol (Eastman Organic Chemicals) were used to test substrates for sludge acclimation. Phenol (Ring UL-^{14}C), 71.1 mCi/mmol and 110 mCi/mmol (99% purity) (Amersham); aniline (Ring UL-^{14}C), 102 mCi/mmol (98.4% purity) (Amersham); toluene (Ring UL-^{14}C), 5.11 mCi/mmol (>98% purity)(Pathfinder Laboratories); and 2,4-dichlorophenol (Ring UL-^{14}C), 4.43 mCi/mmol (>98% purity)(Pathfinder Laboratories) were used as substrates for the bio-oxidation mineralization assays. Ready-solve Hp/b (Beckman) scintillation fluid was used as the cocktail for liquid scintillation spectrometry on a Tracor 6892 scintillation counter.

High performance liquid chromatography (HPLC) grade ethyl acetate and methanol (both Burdick and Jackson) were used as extractants and delivery solvents for test substrates and as mobile phases for HPLC on a Perkin Elmer Series 2 HPLC, equipped with an LC75 variable wavelength UV/VIS detector.

Analytical Determination of Test Substrates

For phenol, the sludge reaction mixture was treated by sonic oscillation for 5 min and centrifuged. One hundred µL of the supernatant was injected into an HPLC system (Perkin Elmer series 2 liquid chromatograph) equipped with a 4.6 mm X 250 mm C-18/10 column and UV light detector. The elution solvent was 50:50 methanol-water. For toluene and 2,4-dichlorophenol, an equal volume of ethyl acetate was added into the sample to extract substrates. One hundred µL of the ethyl acetate phase was injected into an HPLC system. The column used was the same as for phenol, and the elution solvent was 20:80 methanol-water. The flow rate was 2 mL/min. Wavelengths for detection were 272 nm for phenol and dichlorophenol, 254 nm for toluene. Toluene gas phase analysis was conducted by gas chromatography at IT Corporation.

Determination of Metabolites

Samples were extracted as above with the same volume of ethyl acetate at acid conditions. The extracts were analyzed by HPLC at 254 and 272 nm wavelengths. One-mL fractions were collected and assayed for radioactivity where appropriate.

Sludge Maintenance and Acclimation

Sludge derived from the waste treatment facility of a sulfite wood pulping plant was used as inoculum for establishing all sludge cultures described in this report (see Section 5). For sludge maintained at the University of Tennessee, the fill-and-draw technique was used to develop two-stage sludge cultures. A kraft wood pulping foul condensate wastewater was used as the feed stock for sludge maintenance. Two 300-mL sludge cultures were maintained in shaken 500-mL flasks incubated at room temperature (22° ± 3°C). On a daily basis, the sludge was allowed to settle and 100 mL of each 300-mL culture was wasted. Each feed solution was buffered, and nitrogen and phosphorus supplemented by the daily addition of 20 mg NH_4NO_3, 15 mg K_2HPO_4, and 5 mg Na_2HPO_4 (all Fisher Scientific). The first stage reactor received 100 mL fresh feedstock while the second stage reactor received 100 mL waste liquor from the first stage reactor. The effective sludge age of each culture was 3 days.

Single substrate acclimation was employed to acclimate first and second stage cultures to phenol, toluene, and 2,4-dichlorophenol. On a daily basis substrates were added in H_2O or methanolic solution to yield a daily starting concentration of 40 mg/L toluene and phenol and 10 mg/L 2,4-dichlorophenol during the course of acclimation. On a periodic basis, samples were removed from these sludge cultures (before daily feedstock and substrate addition) and were used to assay bio-oxidation rates of ^{14}C-labeled substrates to which the cultures were acclimated.

IT Corporation maintained large-scale fill-and-draw multiple substrate acclimated sludge cultures (see Section 5) and continuous flow single substrate sludge cultures (see Section 10). Samples from these cultures were analyzed for population, density, and mineralization of substrates during this project.

Measurement of Bacterial Population Densities

Total bacterial densities in sludge cultures were determined using the acridine orange direct counting (AODC) procedure.[86] Ten mL sludge samples were homogenized with a tissue grinder (20 strokes) and diluted 1:1000 in phosphate buffered saline with 0.1% Tween 80 and re-homogenized. A 2-mL subsample was stained with 1% acridine orange for 5 min, filtered through a 0.2-μm Nucleopore filter membrane, and mounted on a microscope slide with a drop of non-fluorescent immersion oil. The filters were examined at 1000X magnification under UV light (overhead mercury lamp illumination) using an American Optical model one-ten microstar microscope. The microscope was equipped with BG12 + K418 excitation and OG515 barrier filters. Green and orange fluorescing cells were counted over 10 random fields, averaged, and converted to numbers of cells/mL original sample.

Phenol, toluene, 2,4-dichlorophenol, and aniline degrading bacteria were determined by spread plate inoculation of diluted sludge samples onto a basal agar medium containing each substrate. The basal medium was formulated with 4.0 g, $NaNO_3$; 1.5 g, KH_2PO_4; 0.5 g, Na_2HPO_4; 0.2 g, $MgSO_4 \cdot 7\,H_2O$; 0.01 g, $CaCl_2 \cdot 2H_2O$; 0.07 g, $FeSO_4$; and 18 g, purified agar per L of distilled water (pH 7.0). Fifty mg/L phenol or aniline and 20 mg/L 2,4-dichlorophenol were added to the medium as sole carbon sources. Toluene was added in the vapor phase during incubation; in some cases toluic acid was incorporated into the medium at a concentration of 50 mg/L. Yeast extract, 10 mg/L, was also incorporated in the medium in some instances to provide growth factors to support auxotrophic populations. Inoculated plates (duplicate or triplicate) were incubated at room temperature for a minimum of 7 days, phenol and aniline; 5 days, toluene; and 4 weeks, dichlorophenol.

Sludge and Enzyme Activity Measurements

Oxygen Uptake --

Activated sludges, acclimated to priority pollutants in two stage reactor systems were harvested by centrifugation and washed twice with 0.1 molar phosphate buffer (pH 7.5) then suspended in the phosphate buffer to make half of the initial volume. 1.5 mL of the cell suspension and 1.5 mL of 0.1 molar phosphate buffer (pH 7.5) containing a substrate was placed in the vessel (total vessel volume, 3.0 mL) for oxygen analyses (Type YSI53: Yellow Springs Instruments, Yellow Spring, Ohio). Final substrate concentrations were as follows: phenol 0.5 mmolar; toluene 2.2 mmolar; 2,4-dichlorophenol 0.06 mmolar; all other substrates 0.33 mmolar. Toluene was directly injected into a vessel. The incubation temperature was kept at 26°C. (Chlorobenzene and 1,4-dichlorobenzene were dissolved in methanol and introduced into the reaction mixture. Methanol concentration in the reaction mixture was 3000 mg/L. This concentration exhibited no inhibition of oxygen uptake). Oxygen uptake rates were corrected for endogenous oxygen uptake.

Preparation of Cell Free Extracts --

Freshly harvested cells were washed twice with 0.1 molar phosphate buffer (pH 7.0) by centrifugation and suspended in the same buffer. The cell suspension was sonicated at 50 W, for 5 min. Cell debris was removed by centrifugation at 25,000 g for 30 min. The supernatant was used as cell extract. Protein concentration was determined by Bio-Rad protein assay; that is, a sample was mixed with 5.0 mL of para-Coumassie protein-binding dye (Bio-Rad Laboratories, Richmond, CA) and incubated for 30 min. The absorbance of dye-sample mixture was measured at 595 nm on a Bausch & Lcmb spectronic 70 spectrophotometer. Absorbance measurements were converted to micrograms of protein per milliliter of reaction mixture by comparison to a standard curve prepared with bovine serum albumin (Sigma Chemical Co., St. Louis, MO).

Enzyme Assays --

All enzyme activities were determined by the use of spectrophotometric methods in silica cuvettes with 10 mm path-length in a Beckman DB recording spectrophotometer. One enzyme unit is defined as the amount of enzyme that catalyses the formation of 1 µmol of the product or the disappearance of 1 µmol of the substrate or cosubstrate per min. Specific activity was calculated as enzyme units per mg protein.

Catechol 1,2-dioxygenase activity was assayed by the modification of Nakazawa method.[87] A cuvette contained 300 µM of phosphate buffer (pH 7.5), 5 µM of catechol and 0.1 mL of cell free extract in a final volume of 3.0 mL. The activity was determined by measuring the increase in optical density of 260 nm due to the formation of product, cis, cis-muconic acid.

Catechol 2,3-dioxygenase activity was assayed by the modification of Nazaki method.[88] A cuvette contained the same components as the catechol 1,2-dioxygenase assay. The increase of absorbance at 375 nm was due to the formation of the product, 2-hydroxymuconic semialdehyde.

Phenol hydroxylase was determined by the modification of Neujahr.[89] A cuvette contained 300 µmol of phosphate buffer (pH 7.5), 5 µmol of phenol, 1 µmol of NADPH and cell free extract in a final volume of 3.0 mL. The decrease of absorbance at 340 nm based on the disappearance of the enzyme's specific cosubstrate was measured.

Salicylate hydroxylase activity was assayed by measuring the decrease of absorbance at 340 nm due to the disappearance of NADH. A cuvette contained 300 µmol of phosphate buffer (pH 7.5), 1 mmol of salicylate, 1 µmol of NADH, 0.1 µmol FAD and cell free extract in a final volume of 3.0 mL.

m-Hydroxybenzoate hydroxylase and p-hydroxybenzoate activities were assayed by measuring the decrease of absorbance at 340 nm due to the disappearance of NADPH.[90] A cuvette contained 300 µmol of phosphate buffer (pH 7.5), 0.6 µmol of m-hydroxybenzoate or 2 µmol of p-hydroxybenzoate, 1 µmol of NADPH, 0.1 µmol of FAD and cell free extract in a final volume of 3 mL.

Protocatechuate 3,4-oxygenase activity was determined by measuring the increase of absorbance at 410 nm due to the formation of products, 2-hydroxy carboxymuconic semialdehyde.[88] A cuvette contained 300 μmol of phosphate buffer (pH 7.5), 0.25 μmol of protocatechuate and cell free extract in a final volume of 3.0 mL.

Mineralization Assays

Flow Through Method --

One mL aliquots of the various substrate acclimated sludge and radiolabeled substrates were placed into 2-mL glass vials with Teflon-lined rubber caps (gas chromatography vials marketed by Fisher Chemical Company) and incubated at room temperature. A diagram of this setup is presented in Appendix F. After incubation the cultures were acidified with 0.2 mL of 2N H_2SO_4. CO_2 gas driven out of the solution by the air flow was trapped on 1 mL of 0.2N KOH. Before the flow gas entered the KOH trap, it was passed through a 3 mm X 30 mm Florisil column for removal of volatile substrates. 0.5 mL of CO_2 trapped KOH was placed into scintillation vials containing 15 mL of Ready-Solv HP/b cocktail (Beckman) and quantitated by liquid scintillation techniques. A sterile control, prepared by adding 0.2 mL of 2N H_2SO_4 into 1 mL of reaction mixture, was used to determine the non-biological mineralization, volatilization, or absorption to the walls of the incubation vessels.

Center Well Method --

Two mL of activated sludge and radiolabeled compounds was added to a 20-mL vial holding a center well,[91] and 0.5 mL of 0.2N KOH was placed into the center well. After incubation, 0.4 mL of 2N H_2SO_4 was introduced to the reaction mixture and the reaction vessel was shaken gently for 1 hr to allow complete absorption of liberated CO_2. Then the center well was taken out from the reaction vessel and placed directly into the liquid scintillation vial containing HP/b (Beckman) cocktail.

Kinetic Interpretations

A fundamental assumption was made that, at the substrate concentrations employed, bio-oxidation kinetics followed a first-order rate process relative to the concentration of the test chemical. It was also assumed that overall kinetics of bio-oxidation were a second-order process, first order in substrate and first order in bacteria. The possibility exists that some experimental results can be interpreted as mixed or fractional reaction order; however, for the interpretation of the results of these studies, a first-order reaction mechanism relative to both substrate and degradative bacterial cells was assumed.

RESULTS

Experimental Calibration of the Bio-oxidation Assay

Using phenol as the test substrate, initial batch studies were conducted to determine the effect of experimental conditions on pollutant bio-oxidation, as measured by mineralization of the ^{14}C-labeled substrate. The time course linearity of phenol mineralization was measured by determining the cumulative oxidation of ^{14}C-phenol to $^{14}CO_2$ by phenol acclimated sludge. Up to an incubation period of 60 hr, a direct relationship between cumulative ^{14}C-phenol mineralized and the length of incubation was observed (Fig. 40). Upward inflections in the mineralization curves are indicative of bacterial population growth on prolonged incubation in the presence of the substrate. Consequently, for further assessment of substrate mineralization rates, incubation times were maintained at $\leq$ 24 hr.

The relationship between sludge bacterial population density and rates of phenol oxidation was determined by serial dilution of concentrated sludge (centrifuged) and incubation of each dilution with an identical concentration of ^{14}C-phenol (120 mg/L). Rates of phenol mineralization increased in a linear relationship to increasing bacterial density of activated sludge (Fig. 41). The slope of the regression line fitted to these data points was 3.0×10^{-13}. Based on the theoretical relationship between bacterial density (enzyme concentration) and biotransformation the slope of this line can be interpreted as a first order rate constant in bacteria with the units μg organism^{-1} hr^{-1}. This is not the same constant as the second order rate constant K_2^{bm} (mL org.$^{-1}$hr^{-1}) which is first order in both substrate concentration and biomass concentration.

The influence of substrate concentration on bio-oxidation rate was determined by adding various concentrations of ^{14}C-phenol to sludge and measuring the amount of substrate mineralized after a 24 hr incubation. Initial experiments indicated no net increase in the rate of phenol mineralization upon increasing substrate concentrations (Fig. 42A). These results were obtained for a first stage bio-reactor sludge population defined as: a fill-and-draw reactor using a foul condensate feed stock and phenol acclimation at 40 mg/L with sludge maintained at a 3 day mean cell residence time. Also noted in this experiment was the absence of any acclimation to the phenol substrate as indicated by phenol bio-oxidation rates essentially constant for unacclimated and 2 week acclimated sludge. These results suggested that the foul condensate feed stock was potentially inhibiting the oxidation of the phenol substrate. This interpretation was reinforced by the demonstration of only low rates of phenol bio-oxidation (ca. 10 μgL^{-1} hr^{-1}). Consequently, an in-line second stage reactor was incorporated into the experimental protocol. This reactor was maintained on the spent feedstock waste from the first stage reactor and also subjected to single substrate acclimation at a concentration equivalent to the first stage reactor. As indicated by Fig. 42B, phenol bio-oxidation rates were consistently one to two orders of magnitude greater for second stage acclimated sludge as compared to first stage acclimated sludge. In addition, a linear increase in phenol oxidation rates was observed up to a phenol concentration of 120 mg/L. Though not conclusive, the results of these experiments offer evidence that, during short term incubation, phenol bio-oxidation is a first order rate process with respect to concentration up to a limiting concentration at which apparent toxicity effects yield a net decrease in oxidation rate. These experiments were not expressly designed to determine reaction order, and the potential exists for a fractional reaction order

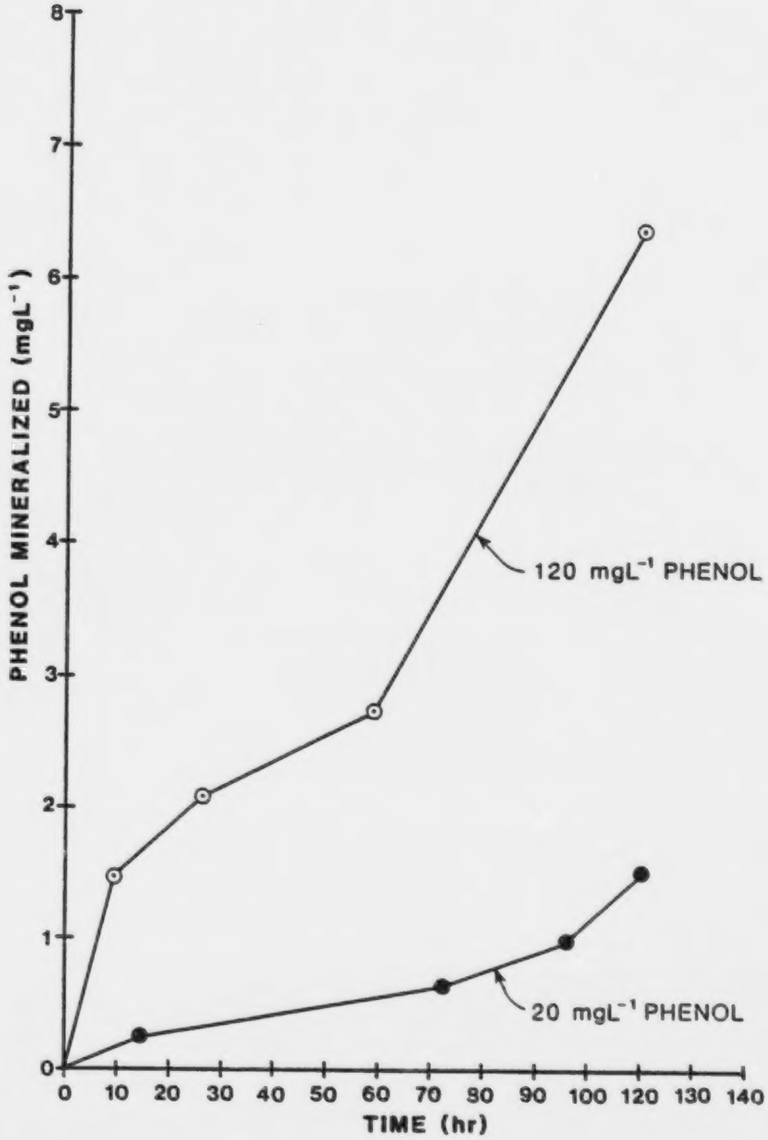

FIG. 40. TIME COURSE MINERALIZATION OF U-^{14}C-PHENOL IN A BATCH REACTOR

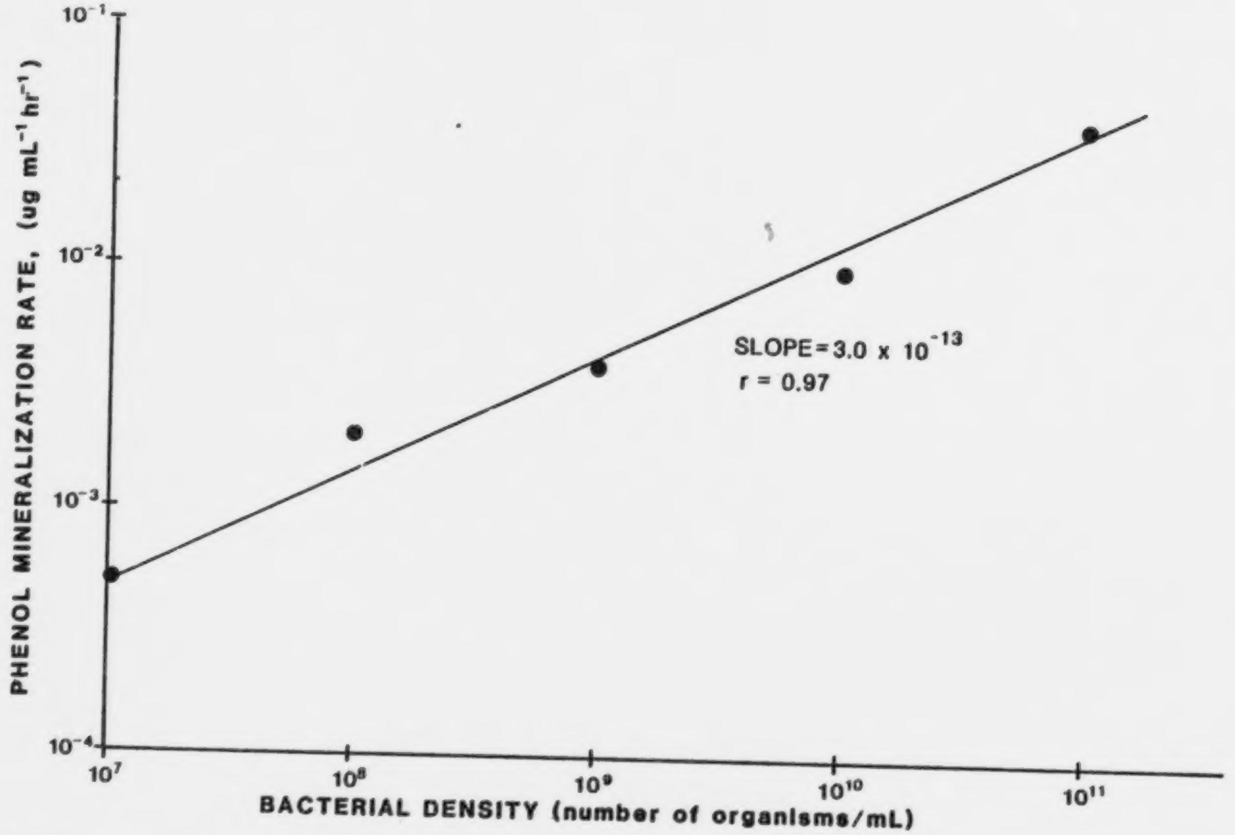

FIG. 41. RELATIONSHIP BETWEEN BACTERIAL POPULATION DENSITIES AND PHENOL MINERALIZATION RATES IN STAGE 1 PHENOL ACCLIMATED SLUDGE

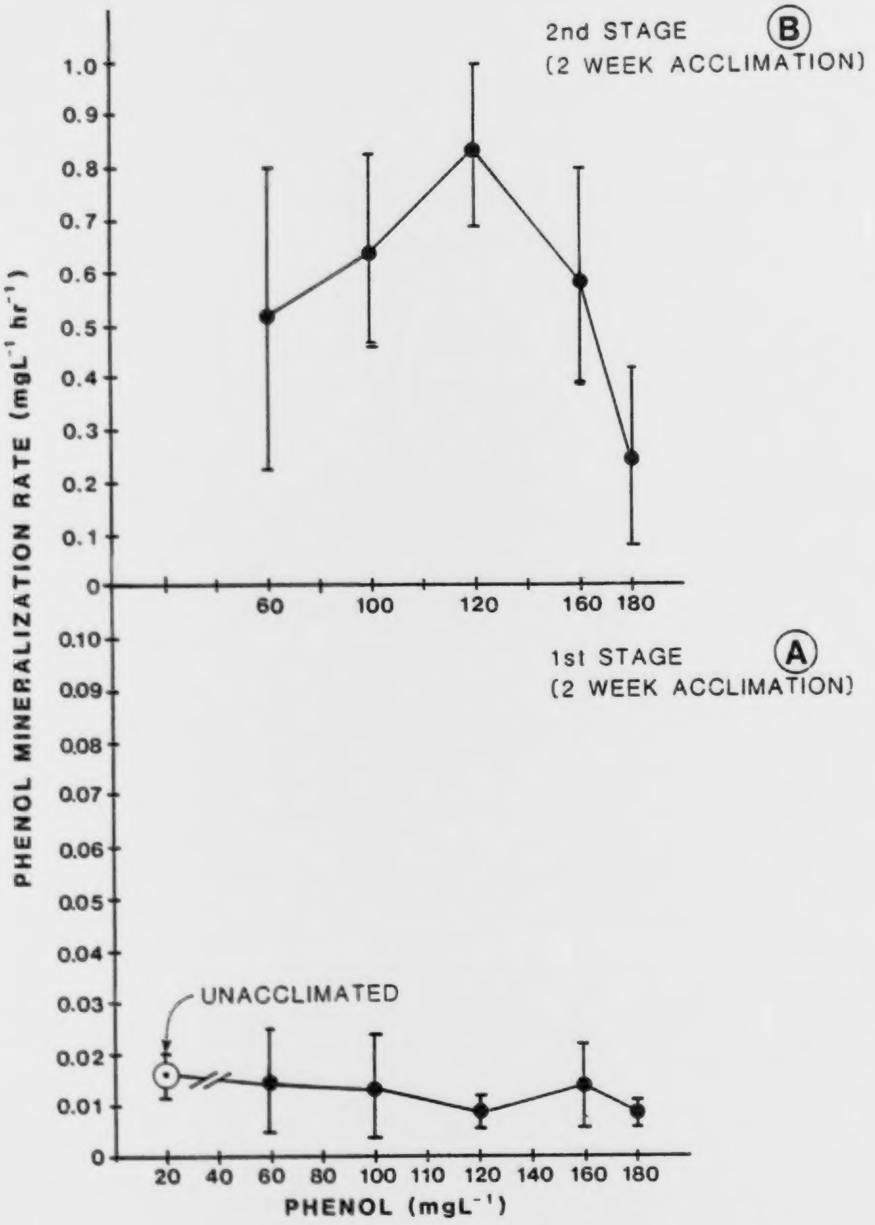

FIG. 42. COMPARATIVE EFFECT OF SUBSTRATE CONCENTRATION ON PHENOL MINERALIZATION RATES IN STAGE 1 AND 2 FILL-AND-DRAW REACTORS

indicative of multiphasic kinetics. Apparent toxicity was observed at higher phenol concentrations (e.g., >120 mg/L) as indicated by decreased rates of phenol mineralization. The bio-oxidation test system developed was then used to measure rates of substrate mineralization (pollutant oxidation) as a function of bacterial population dynamics within activated sludge exposed to single or mixed substrate acclimation.

Biochemical Activity of Sludge Populations

Washed cell sludge culture oxygen uptake rates for six initial test substrates were measured to determine biochemical activity of sludge and potential toxicity of the substrate. The results of these tests, using unacclimated sludge, are given in Table 54. Endogenous oxygen uptake of the sludge and carrier solvent (either water or methanol) was used as a baseline (i.e. 100% 2.2 μLhr^{-1} mL^{-1} incubated for 15 min). Inhibition is indicated by oxygen uptake values less than 100%, while values greater than 100% indicated substrate utilization or increased activity (coefficient of variation, 5%). Phenol, aniline, and toluene demonstrated marginal stimulation or no effect on oxygen uptake at substrate concentrations up to 200 to 300 mg/L. 2,4-dichlorophenol and 1,4-dichlorobenzene, at all concentrations examined, demonstrated increasing toxicity to activated sludge.

TABLE 54. OXYGEN UPTAKE BY NONACCLIMATED SLUDGE EXPOSED TO TEST SUBSTRATES IN A BATCH REACTOR

Concentration (mg/L)	Oxygen uptake (% of endogenous[a])					
	Phenol	Aniline	Toluene	Chlorobenzene	2,4-Dichlorophenol	1,4-Dichlorobenzene
0	100	100	100	100	100	100
10	105	108	100	96	84	77
30	109	98	98	102	61	72
50	90	105	97	88	57	70
100	97	97	95	100	54	60
200	91	92	97	97	16	40
300	98				0	

[a] Endogenous respiration 2.2 μL hr^{-1} mL^{-1} (6.56 μL/hr per 3 mL).

Oxygen requiring enzymatic activity indicative of the biochemical oxidation of a number of key aromatic compounds was measured for unacclimated and phenol acclimated sludge (Table 55). At the substrate concentrations employed, unacclimated sludge demonstrated no oxygen-requiring biochemical activity for any of the test aromatic substrates. For 2 week phenol acclimated sludge, low levels of enzymatic activity were detected for all substrates (Table 55). These results are indicative of either enzymatic induction of oxygenase activity or increased population densities of aromatic, degradative bacteria in the phenol activated sludge.

TABLE 55. COMPARATIVE OXYGEN UPTAKE RATES FOR AROMATIC SUBSTRATES BY UNACCLIMATED AND PHENOL ACCLIMATED SLUDGE IN BATCH REACTORS

Substrate[a]	Oxygen uptake ($\mu g\ hr^{-1}\ mL^{-1}$)	
	Unacclimated	Acclimated[b]
Phenol	0	1.4
Catechol	0	2.1
Salicylate	0	0.1
m-Hydroxybenzoate	0	0.5
p-Hydroxybenzoate	0	1.5
Protocatechuate	0	1.1

[a] 0.5 mMol phenol, all others 0.33 mMol.

[b] Acclimated to 40 mg/L phenol for 2 weeks.

Using Stage 1 and Stage 2 sludge cultures, enzyme assays of crude sludge lysates were conducted for a similar group of test aromatic substrates. Phenol, toluene, and 2,4-dichlorophenol acclimated sludges and unacclimated sludges were examined. Catechol 1,2-oxygenase was the only enzymatic activity observed and this activity was present, in low levels, only in sludges acclimated to the three different test pollutants (Table 56). No detectable enzyme activities for catechol 2,3-oxygenase, phenol hydroxylase, salicylate, m-hydroxybenzoate, p-hydroxybenzoate, or protocatechuate 3,4-oxygenase were observed for either the unacclimated or acclimated sludge cultures (data not shown). It is probable that these enzyme activities are present but below the detectable limits of the enzyme assays employed. It is noted that catechol 1,2-oxygenase activity was on the average two-fold higher in the Stage 1 reactor as compared to the Stage 2 reactors. Such a result indicates either a higher level of enzymatic induction or a greater concentration of bacteria involved in the degradation of aromatic substrates employing a biochemical pathway mediated by the enzyme. This result is somewhat contradictory in that the foul condensate feedstock for the first stage reactor is rich in aromatic substrates and detectable levels of the enzyme would have been expected in the unacclimated sludge.

Based on the above results, phenol hydrolase and catechol oxygenase activity of raw sludge and catechol 1,2-dioxygenase activity of sludge lysates were chosen as standard measures of catabolic enzyme activity in monitoring the bio-oxidative capacity of activated sludge.

TABLE 56. CATECHOL 1,2-OXYGENASE ACTIVITY IN UNACCLIMATED AND ACCLIMATED SLUDGE CULTURES FROM FILL-AND-DRAW REACTORS

Sludge type	Acclimation time	Enzyme activity catechol 1,2-oxygenase (μMol min^{-1} mg protein^{-1})
Unacclimated	0	0
Phenol stage 1	1	0.153
	2	0.145
	3	0.141
	6	
Phenol stage 2	1	0.067
	2	0.046
	3	0.045
	6	
Toluene stage 1	1	0.007
	2	0.012
	3	0.007
	6	
Toluene stage 2	1	0.005
	2	0.005
	3	0.002
	6	
Dichlorophenol stage 1	3	0.045
	5	0.000
	8	0.000
Dichlorophenol stage 2	3	0.021
	5	0.000
	8	0.000

Bio-oxidation in Single Substrate Acclimated Fill-and-Draw Reactors

Phenol Bio-oxidation in Fill-and-Draw Reactors --

Rates of phenol bio-oxidation in fill-and-draw reactors were measured over a 15 week acclimation period. First order rate constants (K_1^{bm})(mineralization rate constant first order for substrate concentration) for ^{14}C-phenol mineralization (units are hr^{-1}) were variable for both first and second stage bio-reactors (Fig. 43). The average K_1^{bm} values for first and second stage reactors were 4.1×10^{-3} hr^{-1} and 1×10^{-2} hr^{-1}, respectively, over the acclimation period. Rate constants for phenol mineralization increased for both reactors during the first 3 weeks of acclimation; it appeared that acclimation was most pronounced in the first stage reactor

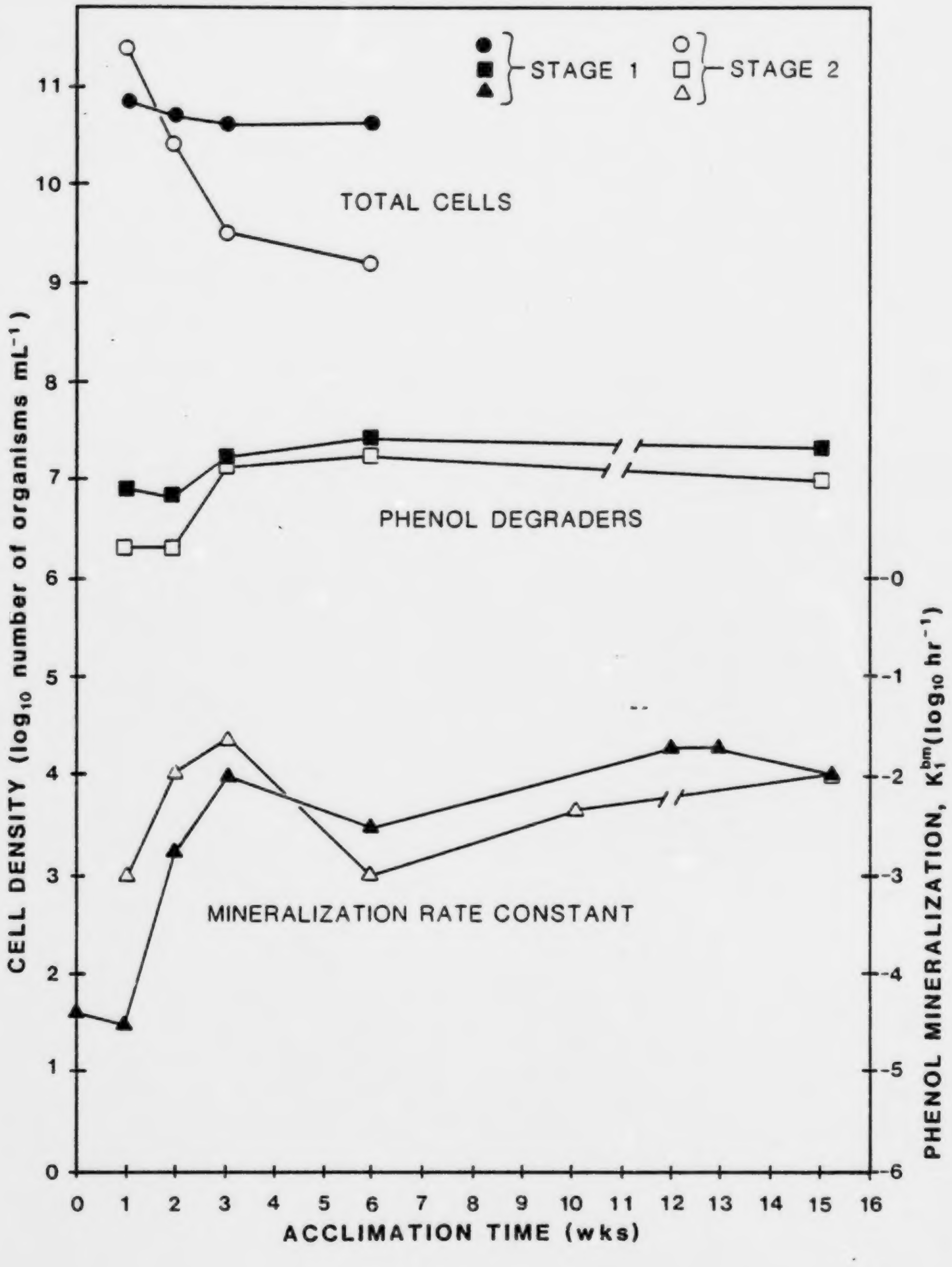

FIG. 43. COMPARATIVE EFFECT OF PHENOL ACCLIMATED TIME ON BACTERIAL POPULATION DENSITIES AND PHENOL MINERALIZATION KINETICS IN FILL-AND-DRAW REACTORS

TABLE 57. BACTERIAL POPULATIONS AND PHENOL MINERALIZATION KINETICS FOR PHENOL ACCLIMATED SLUDGE FROM FILL-AND-DRAW REACTORS

Phenol reactor	Acclimation time[a] (wk)	Total cells (org mL^{-1})	Phenol degraders (org mL^{-1})	Mineralization rate (μg mL^{-1} hr^{-1})	K_1^{bm} (hr^{-1})[b]
Stage 1	1	8×10^{10}	8.3×10^{6}	1.3×10^{-3}	3.1×10^{-5}
	2	6×10^{10}	6×10^{6}	7.9×10^{-2}	2.0×10^{-3}
	3	4×10^{10}	1.7×10^{7}	4.4×10^{-1}	1.1×10^{-2}
	6	3.6×10^{10}	2.5×10^{7}	1.3×10^{-1}	3.0×10^{-3}
	10				
	12			7.3×10^{-1}	1.8×10^{-2}
	13			7.8×10^{-1}	2.0×10^{-2}
	15		1.9×10^{7}	4.2×10^{-1}	1.1×10^{-2}
Stage 2	1	2.8×10^{11}	2.0×10^{6}	4.0×10^{-2}	1.0×10^{-3}
	2	2.5×10^{10}	2.0×10^{6}	5.0×10^{-1}	1.3×10^{-2}
	3	3.0×10^{9}	1.2×10^{7}	9.9×10^{-1}	2.5×10^{-2}
	6	1.6×10^{9}	1.6×10^{7}	4.0×10^{-2}	1×10^{-3}
	10			1.8×10^{-1}	4.5×10^{-3}
	12				
	13				
	15		1.1×10^{7}	3.8×10^{-1}	9.5×10^{-3}

[a] Acclimated at 40 mgL^{-1} phenol.

[b] First order mineralization rate constant.

with an order of magnitude overall increase of K_1^{bm} 3.1 X 10^{-5} to 2.0 X 10^{-2}, as compared to 1 X 10^{-3} to 2.5 X 10^{-2} for the second stage reactor (see Appendix F).

Bacterial populations and enzyme activity were variable during the course of the experiment. Total bacterial densities were approximately equal in both reactors after 1 week acclimation (ca. 1 X 10^{-11} organisms mL^{-1}) (Fig. 43, Table 57). These levels remained relatively constant for the first stage reactor, but declined two orders of magnitude in the second stage reactor over the first 6 weeks of acclimation. Comparatively, phenol degrading bacteria increased in both reactors after 3 weeks of acclimation and remained relatively constant over the remaining acclimation time. However, it was observed that the first stage bio-reactor maintained high densities of phenol-degrading bacteria throughout the remainder of experiment. A similar trend in enzyme activity was observed in the two reactors (Table 58). First stage reactor crude sludge lysates demonstrated two-fold higher levels of catechol 1,2-oxygenase than the second stage reactor. In general, whole sludge oxygen uptake for phenol or catechol was significantly higher in the first stage reactor.

TABLE 58. OXYGEN UPTAKE AND ENZYME ACTIVITIES IN FILL-AND-DRAW REACTORS, PHENOL ACCLIMATED SLUDGE

Phenol reactor	Acclimation time (wk)	Oxygen uptake ($\mu g\ hr^{-1}\ mL^{-1}$)[a] Phenol	Oxygen uptake ($\mu g\ hr^{-1}\ mL^{-1}$)[a] Catechol	Catechol 1,2-oxygenase[b] ($\mu mol\ min^{-1}$ mg $protein^{-1}$)
Stage 1	1	6.5	3.9	0.153
	2	5.4	8.1	0.145
	3	9.0	7.0	0.141
	6	11.2	12.1	
	10			
	12	9.7		
	13	4.5		
	15	10.2		
Stage 2	1	0.8	3.0	0.067
	2	0.8	2.0	0.046
	3	7.4	9.5	0.045
	6	2.0	6.3	
	10			
	12			
	13			
	15	6.1		

[a] Whole sludge.

[b] Crude lysate.

The first stage reactor maintained higher total bacterial densities, phenol degrading bacteria, and higher enzyme activities throughout the course of the experiment; yet rates of phenol mineralization in the first stage reactor were lower than reported for the second stage reactor. These results are indicative of feedstock repression of bio-oxidation in the first stage reactor. In addition, detectable acclimation to phenol was observed in both reactors; yet decreased rates of mineralization were observed during prolonged acclimation. These results suggest a potential methodology problem associated with the bio-oxidation assay system or accumulated phenol toxicity in the single substrate acclimation process. A series of experiments was conducted to analyze the performance of both bio-reactors from the standpoint of feedstock carbon removal and pollutant removal after prolonged acclimation.

After 15 weeks of acclimation, periodic samples were withdrawn from each bio-reactor immediately after feedstock and acclimation-phenol addition. These samples were subjected to analysis for residual phenol. Additional samples, after 24-hr incubation, were entrained in bio-oxidation assay vessels, and dissolved organic carbon (DOC), total CO_2 and $^{14}CO_2$ (from bio-oxidation of a spike of 40 mg/L ^{14}C-phenol) was determined at periodic intervals.

DOC removed from the fill-and-draw reactors over a given batch interval appeared to follow a first order rate decrease of the form $-dC_a/dt = K_1^b C_a$ (Fig. 44A). First order rate constants for DOC removal were calculated from the modified form of this equation ($2.303 \log C_{ao}/C_a = K_1^b t$, where C_{ao} is the initial DOC and C_a is the DOC at time, t). Calculated K_1^b values (hr^{-1}) for the stage 1 and stage 2 reactors were 1.05×10^{-1} and 1.37×10^{-1}, respectively. In comparison, phenol removal rate constants during acclimation in the batch reactor ranged from 2.8×10^{-1} to 1.4×10^{-1} for the first and second stage reactors (Fig. 44C).

As indicated by Fig. 44C, phenol removal from the aqueous phase was essentially complete within 12 and 24 hr for the first and second stage reactors, respectively. This rapid rate of phenol removal demonstrated that there was no residual free phenol in samples used for bio-oxidation analysis. Bio-oxidation assay samples, withdrawn from the semi-continuous reactors after 24-hr incubation and spiked with 40 mg/L phenol, demonstrated initial DOC levels of 40 mg/L carbon (Fig. 44B). DOC removal rate constants in the batch assay reaction mixture were nearly linear through 12 hr incubation, K_1^b (hr^{-1}) approximately 2.15×10^{-1} and 8.7×10^{-2} for the first and second reactor bio-oxidation assay samples, respectively (Fig. 44B). In both samples, DOC increased during the remaining 12 hr of incubation. These results indicate cycling and turnover of cellular carbon. Carbon evolved as CO_2 during bio-oxidation assay incubation accounted for 25% of the initial DOC pool in the system (Fig. 44B). Comparatively, 25% to 29% of ^{14}C-phenol carbon was recovered as CO_2 during the bio-oxidation of phenol (Fig. 44D). In comparing Fig. 44B and D, it is indicated that 90% of carbon recovered as CO_2 during bio-oxidation of the DOC-phenol pool originates from the ^{14}C-phenol carbon and only 10% originates from the DOC pool. Since approximately 9 mg of non-phenol DOC is present in the reaction mixture, carbon

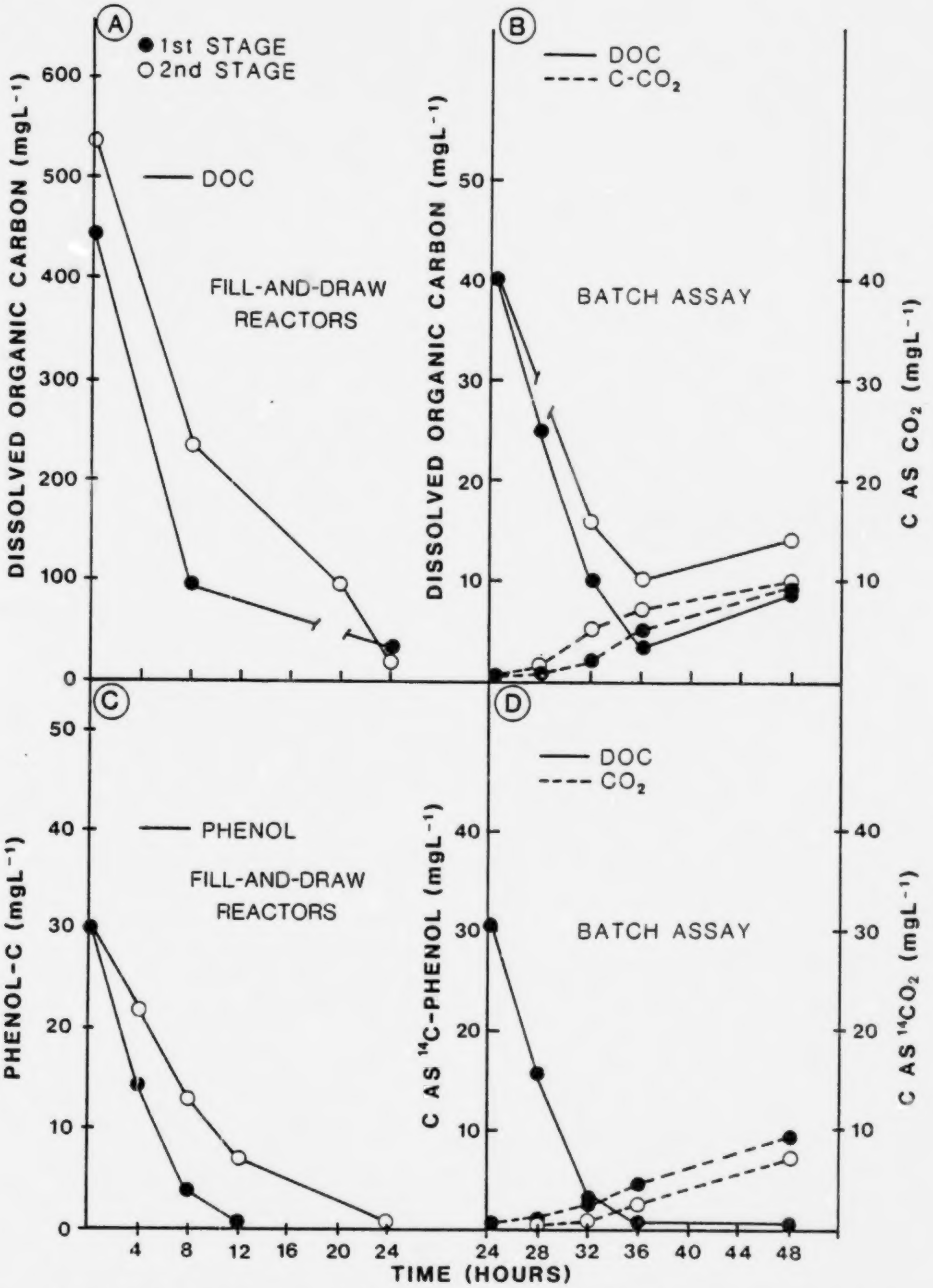

FIG. 44. KINETICS OF CARBON AND PHENOL REMOVAL FROM BOTH FILL-AND-DRAW ACCLIMATION AND BIO-OXIDATION ASSAY REACTORS

dioxide liberated from this fraction represents approximately 10% of the non-phenol DOC. However, it is observed that both phenol carbon and ^{14}C-phenol carbon in solution are completely removed from solutions within 12 hr in both the stage 1 reactor and the bio-oxidation samples (Fig. 44C and D). These results indicate residual carbon in the reaction mixture is non-phenol and that non-phenol DOC removal is minimal and can be accounted for entirely by CO_2; i.e., non-phenol DOC removal is accomplished via 100% mineralization.

In a separate group of experiments a ^{14}C-phenol material balance was performed for bio-oxidation assay samples. Total recovery of radioactivity in distilled water and sterile sludge (acidified) control assays ranged from 99.3 to 100% (Table 59). In experimental samples, total recovery was found to be approximately 77%. The 13% loss of radioactivity is most likely associated with phenol assimilation and biomass quenching of radioactivity detection. In the future, this may be avoided with the use of a tissue digestion solvent or a thermal biological oxidizer. Up to 43.6% of the ^{14}C-phenol was respired as CO_2; while approximately 33% of the radioactivity was associated with the residual reaction mixture. This residual can include metabolites, biomass, and parent phenol. As was indicated in Figure 44C, parent phenol is generally not detected after 24 hr in either the first or second stage reactors. However, significant production of radioactive transformation products (direct metabolites or products of biomass decomposition) can account for up to 25% of the radioactivity in solutions (Table 60). However, less than <10% of the radioactivity is associated with transformation production after 24-hr incubation of bio-oxidation reaction mixture. These results, in part, explain the accumulation of DOC during the latter half of incubation during the assay (Fig. 44B).

TABLE 59. MATERIAL BALANCE FOR PHENOL BIO-OXIDATION BATCH ASSAY

		Percent recovered as			
Sample type	Incubation time (hr)	CO_2	Volatile	Reaction vessel residual	Total recovery
Distilled H_2O	24	0.4	13.0	92.6	106.0
Sterile control (acidified)	8	2.2	6.8	91.6	100.6
	24	2.9	13.6	82.8	99.3
Acclimated sludge	8	30.3	4.9	41.7	76.9
	24	43.6	2.1	32.1	77.8

TABLE 60. TIME COURSE DISTRIBUTION OF RADIOACTIVITY BETWEEN PHENOL AND TRANSFORMATION PRODUCTS DURING BIO-OXIDATION BATCH ASSAY

Incubation time (hr)	Distribution of radioactivity in solution (%) Phenol	Metabolites
0	100	0
4	50	20
8	10	25
12	0	20
24	0	10

Growth of phenol degrading bacteria, during the course of incubation, in the bio-oxidation assay was observed (Table 61). Three- to four-fold increases in phenol degrading bacteria were observed in the first and second stage reactors, respectively. This increase was detected within 8 hr in assay samples from the first stage reactor and correlates with observed mineralization rates which were maximum at 4 hr incubation. Population growth in the second stage reactor was maximized at 24-hr incubation and correlated with increasing rates of mineralization on prolonged incubation. For both reactors, 0 time and 24-hr incubation measurements of oxygen uptake on the phenol substrate increased in proportion to the overall increased phenol degrading bacteria (2- and 6-fold, respectively).

TABLE 61. COMPARATIVE TIME COURSE RELATIONSHIP BETWEEN PHENOL BIODEGRADATION AND BACTERIAL POPULATIONS AND ACTIVITY IN FILL-AND-DRAW REACTORS

Phenol reactor	Incubation time (hr)	Phenol residual (mgL^{-1})	Phenol degradative bacteria ($org.\ mL^{-1}$)	Oxygen uptake ($mgL^{-1}\ hr^{-1}$)	Mineralization rate ($mgL^{-1}\ hr^{-1}$)
Stage 1	0	40	9.7×10^6	7.9	
	4	19			0.61
	8	5	3.0×10^7		0.46
	12	1			0.46
	24	0	1.9×10^7	14.6	0.42
Stage 2	0	40	2.6×10^6	1.4	
	4	28			0.05
	8	17	9.3×10^6		0.19
	12	9			0.28
	24	0.7	1.1×10^7	8.7	0.38

Toluene Bio-oxidation in Fill-and-Draw Reactors --

Toluene bio-oxidation was examined in an experimental format similar to phenol oxidation; however, preliminary assay calibration experiments conducted using a phenol substrate were not performed for toluene or 2,4-dichlorophenol, a later substrate.

Time course toluene acclimation (40 mg/L) demonstrated an early enhanced acclimation to toluene in a second stage acclimated reactor which demonstrated 20-fold higher rates of toluene mineralization when compared to the first stage reactor (Fig. 45). A similar response was previously demonstrated for phenol (Fig. 43). However, a severe perturbation in the second stage reactor occurred after 3 weeks acclimation resulting in virtually undetectable levels of toluene mineralization. After 6 weeks incubation, the sludge population recovered and again promoted elevated levels of toluene mineralization. This perturbation in toluene mineralization in the second stage reactor was also reflected in 3- to 4-fold decreases in both total bacteria and phenol degrading bacteria comprising the sludge population. Both populations recovered or stabilized and were essentially quantitatively identical to the comparable population in the second stage reactor.

During the course of acclimation to toluene, material balance studies indicated that the flow through bio-oxidation assay was severely limited in efficiency for toluene due to volatilization of the substrate (see below). Consequently, a static assay (centerwell method) in which the CO_2 was collected in an alkali center well trap internal to the reaction vessel was used as a modification of the flow through system. Under these conditions, 20-week toluene acclimated sludge, challenged with 130 mg/L, ^{14}C-toluene, demonstrated a kinetic mineralization rate constant of 1.1×10^{-2}. These results are approximately 2 orders of magnitude higher than the previous (1 to 10 week acclimation) results for toluene in the stage 1 reactor and three fold higher than any result obtained for the stage 2 reactor. Consequently, rate estimates for toluene bio-oxidation in semi-continuous reactors during the first 10 weeks of acclimation are not quantitative and can only be used as a qualitative response of the sludge during acclimation.

Although total bacterial concentrations in the toluene bio-reactors (Table 62) were similar to the phenol reactors, toluene degrading bacteria were maintained at levels an order of magnitude less than those reported for the phenol reactors (Table 57). It should be noted that on prolonged incubation (20 weeks), an approximate 5-fold increase in toluene-degrading bacteria was detected in both toluene reactors. These results, in part, contribute to the significant increase observed in oxygenase activity of toluene acclimated sludge (Table 63). As indicated by Tables 62 and 63, oxygenase activity was observed in toluene acclimated sludge with greater than 6.5×10^5 toluene degrading bacteria mL^{-1}. These results may set the lower level of detection for this assay and directly influence its utility in monitoring the bio-oxidative capacity of various sludges.

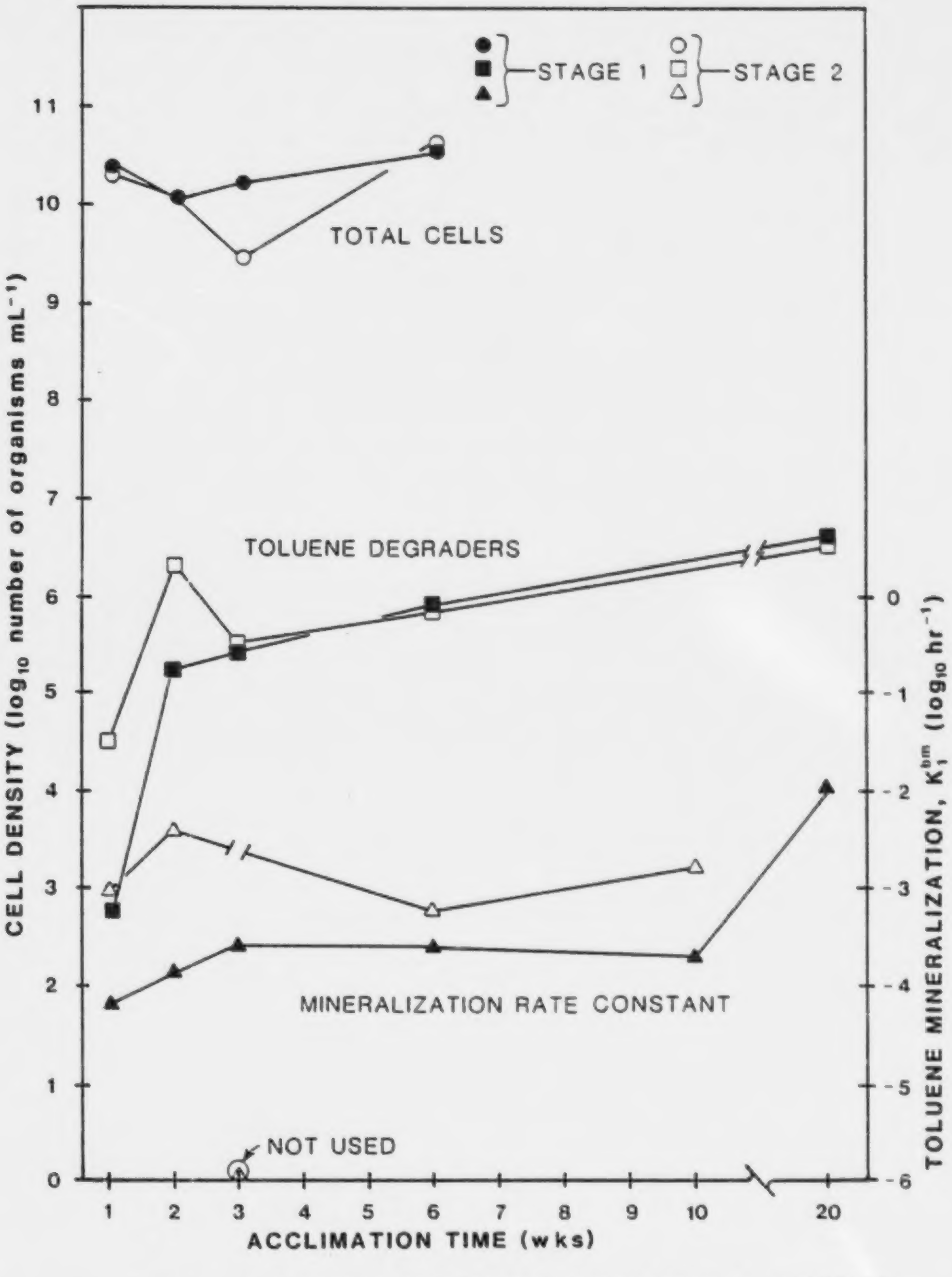

FIG. 45. COMPARATIVE EFFECT OF TOLUENE ACCLIMATION ON BACTERIAL POPULATION DENSITIES AND TOLUENE MINERALIZATION KINETICS IN FILL-AND-DRAW REACTORS

TABLE 62. BACTERIAL POPULATIONS AND TOLUENE MINERALIZATION KINETICS IN FILL-AND-DRAW REACTORS, TOLUENE ACCLIMATED SLUDGE

Toluene reactor	Acclimation time (wk)[a]	Total cells (org. mL^{-1})	Toluene degraders (org. mL^{-1})	Mineralization rate ($\mu g\ mL^{-1}\ hr^{-1}$)	K_1^{bm} (hr^{-1})
Stage 1	1	3×10^{10}	6.0×10^{2}	2.3×10^{-3}	5.7×10^{-5}
	2	1×10^{10}	1.6×10^{5}	6.6×10^{-3}	1.7×10^{-4}
	3	2×10^{10}	2.5×10^{5}	8.3×10^{-3}	2.0×10^{-4}
	6	4.1×10^{10}	7.2×10^{5}	7.0×10^{-3}	1.8×10^{-4}
	10			7.0×10^{-3}	1.7×10^{-4}
	20[b]		3.6×10^{6}	1.4×10^{0}	1.1×10^{-2}
Stage 2	1	2.3×10^{10}	3.0×10^{4}	4.1×10^{-2}	1.0×10^{-3}
	2	8.8×10^{9}	1.8×10^{6}	1.5×10^{-1}	3.7×10^{-3}
	3	3.0×10^{9}	3.5×10^{5}	$<1 \times 10^{-4}$	
	6	4.5×10^{10}	6.5×10^{5}	3.5×10^{-2}	8.8×10^{-4}
	10			5.0×10^{-2}	1.3×10^{-3}
	20		3.1×10^{6}		

[a] Acclimation at 40 mg/L toluene.

[b] Indicates bio-oxidation assay conducted at 200 mg/L toluene.

TABLE 63. OXYGEN UPTAKE AND ENZYME ACTIVITIES IN FILL-AND-DRAW REACTORS, TOLUENE ACCLIMATED SLUDGE

Toluene reactor	Time (wk)	Oxygen uptake ($\mu g\ hr^{-1}\ mL^{-1}$)[a]		Catechol 1,2-oxygenase[b] ($\mu Mol\ min^{-1}$ mg $protein^{-1}$)
		Phenol	Catechol	
Stage 1	1	0	0	0.007
	2	0	0	0.012
	3	0	0	0.007
	6	0.8		
	10			
	20	3.5		
Stage 2	1	0	0	0.005
	2	1.6	1.9	0.005
	3	0	0	0.002
	6	0.2		
	10			
	20	0.4		

[a] Whole sludge.

[b] Crude lysate.

A mass balance calculation for toluene metabolism in the flow through assay system demonstrated a 35% recovery of the ^{14}C-toluene substrate. This mandated the use of the centerwell assay system. As indicated by Table 64, approximately 80% of the added radioactivity in the sterile system was recovered while approximately 73% was recovered in the experimental system. Of this quantity, 13.7% was accounted for as mineralized substrate ($^{14}CO_2$) after 24 hr incubation. The initial toluene concentration in this assay was 130 mg/L as adjusted for an initial 35% loss of toluene due to volatilization upon sample substrate addition.

TABLE 64. MATERIAL BALANCE FOR TOLUENE STATIC BIO-OXIDATION ASSAY[a]

		Percent recovered as		
Sample type	Incubation time (hr)	CO_2	Residual toluene (vessel and volatile)	Total recovery
Sterile control	4	2.5	74.0	76.5
	24	2.9	78.3	81.0
Reactor 1	4	3.5	70.4	73.9
	24	13.7	59.6	73.3

[a] 20 week toluene acclimation at 40 mg/L.

Dichlorophenol Bio-oxidation in Fill-and-Draw Reactors --

Mineralization of 2,4-dichlorophenol was observed in bio-oxidation assay samples from both first and second stage reactors (Fig. 46). Maximum rates of mineralization, achieved after 5 week acclimation with 40 mgL^{-1} hr^{-1}, were 0.027 and 0.049 mgL^{-1} hr^{-1} for stage 1 and 2 reactors, respectively (Table 65). No detectable mineralization of the substrate was observed at 8 week acclimation. Total cell densities decreased from approximately 1 X 10^9 org. mL^{-1}. Degradative cells were marginally above the background of control agar medium without the 2,4-dichlorophenol carbon source (i.e., less than 5% higher) and no quantitative significance could be established from these numbers. Marginal oxygen uptake activity was detected in the sludge culture after 3 week acclimation with no detectable uptake demonstrated after 5 and 8 week acclimation (Table 66).

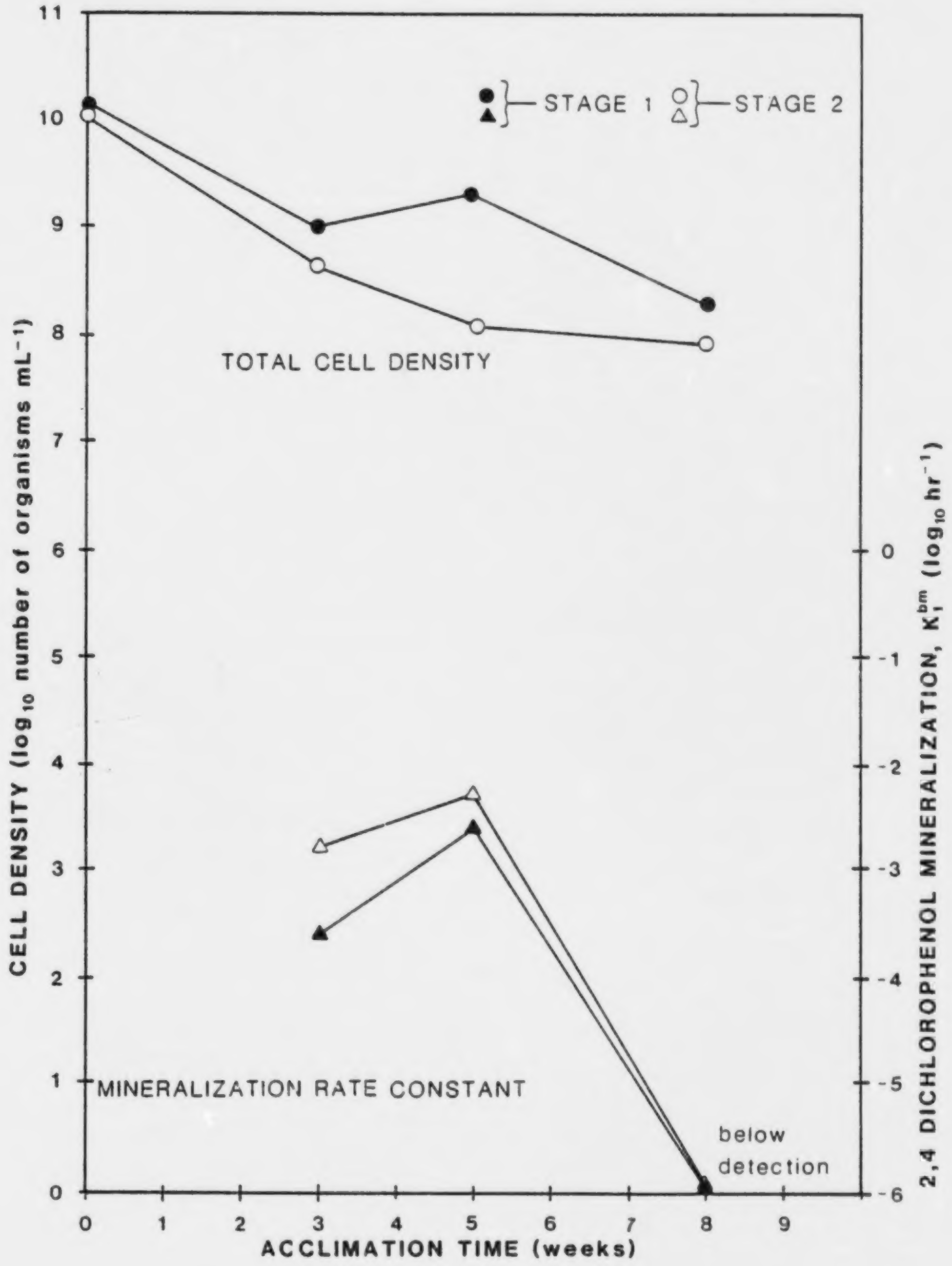

FIG. 46. MICROBIAL RESPONSE TO 2,4-DICHLOROPHENOL ACCLIMATION IN FILL-AND-DRAW REACTORS

TABLE 65. BACTERIAL POPULATION AND 2,4-DICHLOROPHENOL MINERALIZATION KINETICS IN FILL-AND-DRAW REACTORS, DICHLOROPHENOL ACCLIMATED SLUDGE

Reactor	time (wk)	cells (org. mL^{-1})	degraders (org. mL^{-1})	rate ($\mu g\ mL^{-1}\ hr^{-1}$)	K_1^{bm} (hr^{-1})
Stage 1	3	1.0×10^9	$<10^3$	2.2×10^{-3}	2.2×10^{-4}
	5	2.0×10^9	$<10^3$	2.7×10^{-2}	2.7×10^{-3}
	8	2.2×10^8	$<10^3$	$<10^{-4}$	
Stage 2	3	4.0×10^8	$<10^3$	1.5×10^{-3}	1.5×10^{-3}
	5	1.0×10^8	$<10^3$	4.9×10^{-2}	4.9×10^{-3}
	8	8.0×10^8	$<10^3$	$<10^{-4}$	

TABLE 66. OXYGEN UPTAKE AND ENZYME ACTIVITIES IN FILL-AND-DRAW REACTORS, 2,4-DICHLOROPHENOL ACCLIMATED SLUDGE

Reactor	Acclimation time (wk)	Oxygen uptake ($\mu g\ hr^{-1}\ mL^{-1}$)[a]		Catechol 1,2-oxygenase[b] ($\mu Mol\ min^{-1}$ mg $protein^{-1}$)
		Phenol	Catechol	
Stage 1	3	1.6	0.45	0.040
	5	0		
	8	0	0	
Stage 2	3	0.6	4.1	0.021
	5	0		
	8	0	0	

[a] Whole sludge.

[b] Crude lysate.

Both the loss of total cells during acclimation and undetectable dichlorophenol mineralization after 8 weeks indicated a toxic perturbation of dichlorophenol shock in the bio-reactors. This prompted an analysis of residual dichlorophenol level in the two reactors. This analysis demonstrated a three-fold greater level of residual dichlorophenol in the sludge filtrates for both reactors after 9 week acclimation as compared to 6 week acclimation (Table 67). Two-fold higher levels of the substrate were found in the sludge biomass over the same time interval. These results, obtained 24 hr after daily feeding of the sludge, indicate both incomplete removal of the substrate and accumulation of the substrate to a concentration sufficient to induce the toxic perturbations shown by Fig. 46. This interpretation is further supported by earlier results demonstrating inhibition of oxygen uptake of sludge exposed to dichlorophenol concentrations similar to those that accumulated in the reactor. From these results and the failure to demonstrate a significant dichlorophenol degrading population in the sludge, it was decided that dichlorophenol bio-oxidation studies would be discontinued in further research using continuous flow reactors. This was deemed appropriate since no data or conclusions could be obtained on the relationship between microbiological population and the kinetics of substrate bio-oxidation.

TABLE 67. ACCUMULATION OF RESIDUAL 2,4-DICHLOROPHENOL DURING FILL-AND-DRAW REACTOR ACCLIMATION

		2,4-Dichlorophenol (mgL^{-1})	
Reactor	Acclimation (wk)	Sludge supernatant	Sludge biomass
1	6	12	26
	9	33	65
2	6	9	14
	9	28	28

Bio-oxidation in Continuous Reactors

Continuous reactor sludge cultures (IT Corporation) were maintained on foul condensate and a single test compound. Sludge, inoculum for these reactors, was developed by semi-continuous multiple substrate acclimation for a period of 5 months prior to the seeding of the continuous reactors. Details of the maintenance and sampling of the continuous reactors are discussed in Chapter 10.

Substrates examined in continuous reactor studies include phenol, toluene, aniline, and pentachlorophenol. Sludge from these reactors was used to conduct phenol bio-oxidation assays using the flow through protocol as described for batch reactors. Toluene and aniline bio-oxidation assays used the centerwell protocol as described. Mineralization rates, total cells, and degradative bacteria were monitored over time for two reactors maintained at mean cell residence times of approximately 5 and 10 days for each substrate run. The results of these experiments are summarized in Table 68 and graphically described in Figs. 47 through 49.

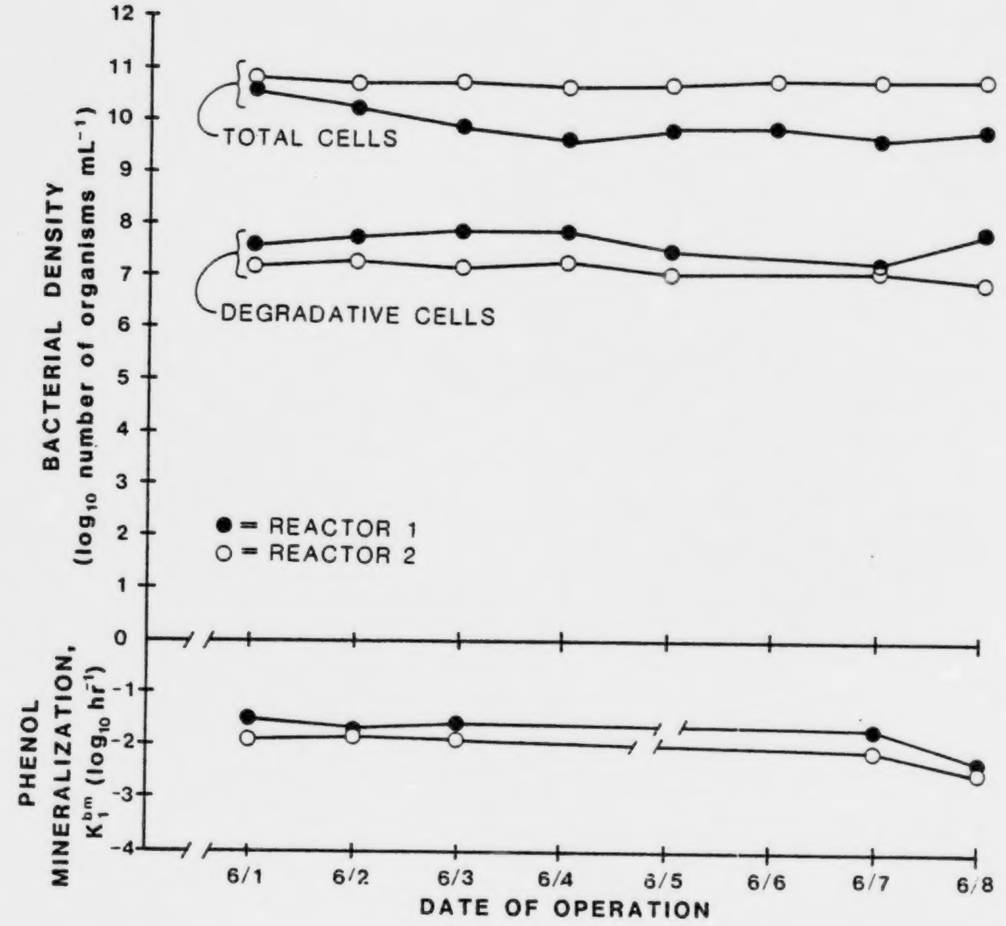

FIG. 47. COMPARATIVE BACTERIAL DENSITIES AND PHENOL MINERALIZATION KINETICS IN CONTINUOUS FEED PHENOL BIOREACTORS

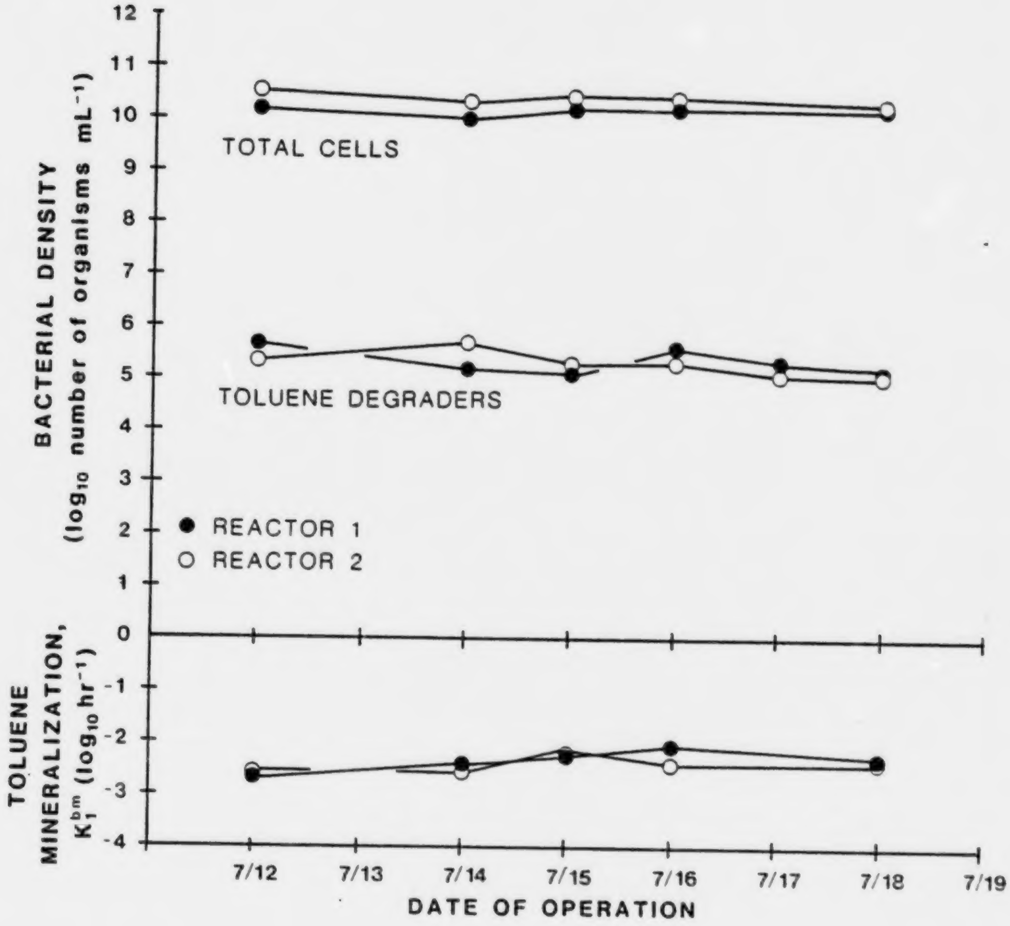

FIG. 48. COMPARATIVE BACTERIAL POPULATION DENSITIES AND TOLUENE MINERALIZATION RATES IN CONTINUOUS FEED TOLUENE BIOREACTORS

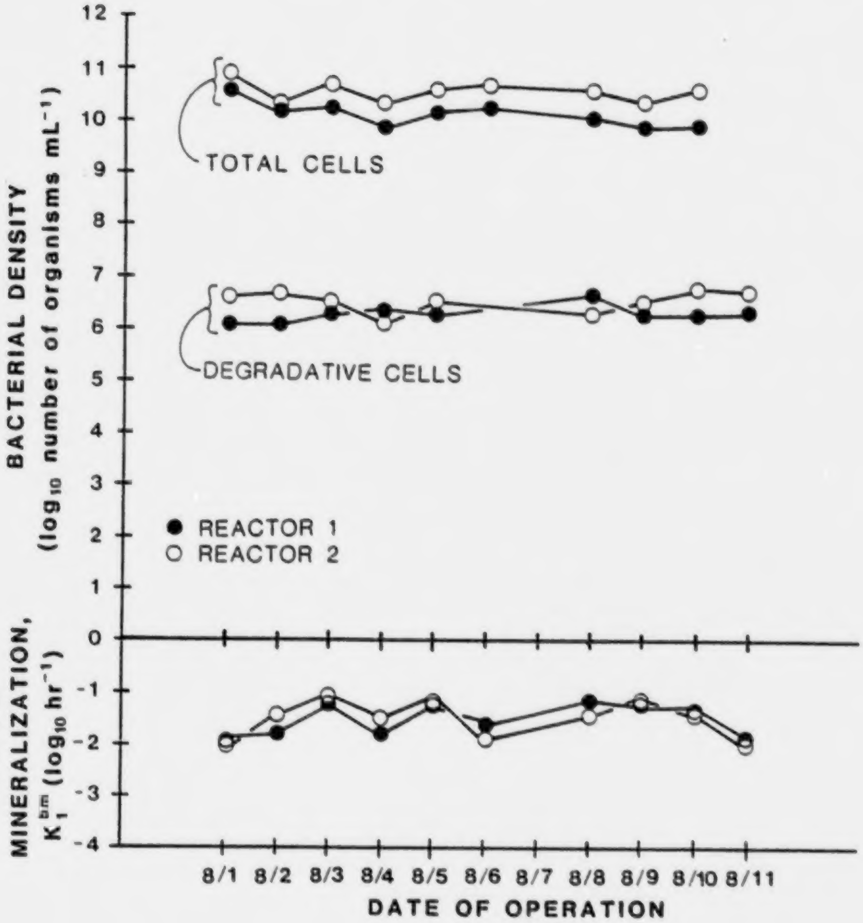

FIG. 49. COMPARATIVE BACTERIAL POPULATION DENSITIES AND ANILINE MINERALIZATION KINETICS IN CONTINUOUS FEED ANILINE BIOREACTORS

TABLE 68. MINERALIZATION RATE CONSTANTS IN CONTINUOUS FEED BIOREACTORS

Substrate	Bio reactor[b]	Mean[a] kinetic rate constant	
		K_1^{bm} (hr^{-1})	K_2^{bm} (mL org^{-1} hr^{-1})
Phenol	1	$1.9 \times 10^{-2} \pm 8.8 \times 10^{-3}$	$5.5 \times 10^{-10} \pm 2.8 \times 10^{-10}$
	2	$1.0 \times 10^{-2} \pm 4.7 \times 10^{-3}$	$6.0 \times 10^{-10} \pm 2.0 \times 10^{-10}$
Toluene	1	$4.8 \times 10^{-3} \pm 2.3 \times 10^{-3}$	$2.5 \times 10^{-8} \pm 1.5 \times 10^{-8}$
	2	$4.2 \times 10^{-3} \pm 2.2 \times 10^{-3}$	$2.6 \times 10^{-8} \pm 1.4 \times 10^{-8}$
Aniline	1	$4.1 \times 10^{-2} \pm 2.5 \times 10^{-2}$	$1.9 \times 10^{-8} \pm 1.2 \times 10^{-8}$
	2	$4.0 \times 10^{-2} \pm 2.5 \times 10^{-2}$	$1.6 \times 10^{-8} \pm 1.1 \times 10^{-8}$

[a] Mean of 5,5, and 9 observations (days) for phenol, toluene, and aniline, respectively (triplicate determinations per observation).

[b] Bioreactors No. 1 and 2 operated at a nominal mean cell residence times of approximately 5 and 10 days, respectively.

Phenol Bio-oxidation in Continuous Flow Bio-Reactors --

Phenol mineralization was determined at five intervals over a period of 1 week (Fig. 47). Mean rates of phenol mineralization (at an assay phenol concentration of 40 mg/L were 0.75 and 0.40 mgL^{-1} hr^{-1} for the two reactors. The highest rate of phenol oxidation (1.15 mgL^{-1} hr^{-1}) was demonstrated on June 1, the first sample time for mineralization assessment. In general, mineralization rates decreased five-fold during the course of the experiment. Mean k_1^{bm} values for phenol oxidation were similar and of the same magnitude as results reported for samples from batch reactors during phenol acclimation (Table 57).

Phenol degradative cells were maintained in the continuous reactor at levels between 1×10^7 and 1×10^8 org. mL^{-1} and were higher but of the same magnitude as reported for batch reactors. Total cells were maintained at levels greater than 2×10^{10} org. mL^{-1} in reactor 2 and, after an initial decrease, 5×10^9 org. mL^{-1}, in reactor 1. These results are also higher but of the same magnitude as samples from batch reactors.

Toluene Bio-oxidation in Continuous Flow Bio-Reactors --

Toluene bio-oxidation assays of sludge samples from continuous reactors were conducted at an effective substrate concentration of 130 mg/L. Mean mineralization rates for toluene were 0.63 mgL^{-1} hr^{-1} and 0.55 mgL^{-1} hr^{-1} for the duplicate reactors with an overall range of 0.31 mgL^{-1} hr^{-1} to 1.02 mgL^{-1} hr^{-1} (Table 68). Approximate mean k_1^{bm} for toluene ranged from 4.8×10^{-3} to 4.1×10^{-3} (hr^{-1}) under the assay conditions employed (Table 68). The bio-oxidation response of the two reactors was virtually identical (Fig. 48). Mineralization rate constants were similar to those obtained for toluene mineralization in batch reactors acclimated 20 weeks to toluene (Fig. 45). Total cell densities for the two reactors ranged from

1.5×10^{10} org. mL^{-1} and 3.4×10^{10} org. mL^{-1} (Table 68 and Fig. 48) similar to but with less variability than observed in fill-and-draw toluene acclimation (Fig. 45). Toluene degrading bacteria, which were non-detectable in the seed inoculum, were maintained at concentrations greater than 1×10^5 org. mL^{-1} throughout the course of the experiment and were approximately an order of magnitude lower than semi-continuous reactors acclimated to toluene for 20 weeks. In all cases, at termination of the experiment, both reactors 1 and 2 showed identical levels of total cells and total degradative cells, and demonstrated identical toluene mineralization kinetics.

Aniline Bio-oxidation in Continuous Flow Bio-Reactors --

Aniline demonstrated the most rapid rates of bio-oxidation of the three test substrates used in continuous reactor studies (Table 68). At a concentration of 100 mg/L the two reactors demonstrated mean rates of mineralization of 4.1 mgL^{-1} hr^{-1} and 4.0 mgL^{-1} hr^{-1}. The response of both reactors was essentially identical with overall mineralization rates ranging from 1.1 mgL^{-1} hr^{-1} to 7.4 mgL^{-1} hr^{-1} (Fig. 49). Total bacterial populations were maintained between 8×10^9 org. mL^{-1} and 9.1×10^{10} org. mL^{-1} in the two reactors with reactor 2 consistently demonstrating higher total cell densities. Aniline degradative populations were maintained at $>1 \times 10^6$ org. mL^{-1} in both reactors. The degradative cell densities were intermediate between phenol and toluene degradative cell densities observed in the two previous continuous reactor experiments.

Comparison of Bio-oxidation Kinetics--

Sludge cultivation using fill-and-draw reactors was performed for the purpose of establishing acclimated sludge used in developing the bio-oxidation assay and to determine the bio-oxidative response of the sludge during acclimation to test pollutant substrates. Continuous feed sludge cultures were employed to determine the contribution of bio-oxidation to overall pollutant fate assessment in simulated waste treatment processes. To bring unity to these two groups of bio-oxidation experiments, a comparison was made among substrate mineralization kinetics for the two sludge (bio-reactor) types. For the purposes of this comparison, mineralization rate constants (K_1^{bm}), first order for substrate concentrations, were used to compare overall sludge performance independently of bacterial densities. Second order mineralization rate constants K_2^{bm} (mL org.$^{-1}$hr^{-1}) were derived from the relationship, $-r^b = K_2^{bm} C_a X$; where $-r^b$ is the rate of mineralization of the substrate C_a and X is the concentration of degradative bacterial cells for the specific substrate.[92] Degradative bacterial cells were chosen as the rate-limiting variable since it is this fraction of the total bacterial density in sludge that is responsible for mineralization of the substrate. These second order rate constants were used in the comparative assessment and prediction of the bio-oxidation fate of the test substrates.

An overall summary of these rate constants for the substrates examined is given in Table 69. There was less than a two-fold difference in the maximum attained first order rate constant for phenol mineralization in either batch or continuous sludge cultures. This relationship also holds true for toluene mineralization in the same comparison. A like relationship

TABLE 69. COMPARISON BETWEEN SUBSTANCE MINERALIZATION RATE ASSAY FOR FILL-AND-DRAW AND CONTINUOUS FEED BIO-REACTOR SLUDGE CULTURES

Substrate	Fill-and-draw sludge culture				Continuous sludge culture			
	Maximum[a]		Mean[b]		Maximum		Mean	
	K_1^{bm}	K_2^{bm}	K_1^{bm}	K_2^{bm}	K_1^{bm}	K_2^{bm}	K_1^{bm}	K_2^{bm}
Phenol	2.5×10^{-2}	2.1×10^{-9}	8.8×10^{-3}	2.5×10^{-10}	2.9×10^{-2}	6.5×10^{-10}	1.6×10^{-2}	2.1×10^{-10}
Toluene	1.1×10^{-2}	3.1×10^{-9}	2.2×10^{-3}	2.9×10^{-9}	7.8×10^{-3}	4.3×10^{-8}	4.5×10^{-3}	1.1×10^{-9}
Aniline					7.0×10^{-2}	3.6×10^{-8}	4.3×10^{-2}	2.6×10^{-9}
Dichlorophenol	2.7×10^{-3}							

[a] Calculated from individual observations at the maximum attained rate of substrate mineralization.

[b] Derived from regression analysis of all data points.

is observed for the mean first order rate constants for the two substrates in the different sludge cultures. It is observed that maximum K_1^{bm} values are four- to five-fold higher than mean K_1^{bm} values in fill-and-draw sludge cultures; while in continuous sludge cultures maximum K_1^{bm} values are approximately two-fold higher than mean K_1^{bm} values for the two substrates. This difference is attributed to the acclimation process in the fill-and-draw reactors; while the continuous reactors employed sludge preacclimated to the substrates prior to bio-oxidation assessment. These results are indicative of the quantitative role of developing bacterial populations during acclimation and can be reflected in second order mineralization rate constants. More emphasis on this issue is found in Section 10.

During fill-and-draw culture conditions, maximum K_2^{bm} values for toluene and phenol were similar, indicating approximately equal rates of conversion of substrate to CO_2 on a per cell basis. However, mean K_2^{bm} values under fill-and-draw conditions indicate a ten-fold higher per cell rate of substrate mineralization are predicted for toluene degrading cells as compared to phenol degrading cells. This relationship is reinforced in continuous culture experiments which demonstrate 1 to 1.5 order of magnitude greater K_2^{bm} values for toluene as compared to phenol. The greater proportion of mineralization of toluene as compared with phenol suggests that while phenol is rapidly removed from the reaction mixture (Fig. 44C and D), the substrate is used as a carbon source to support growth to a greater extent than toluene. As a corollary hypothesis, toluene and aniline (which demonstrates the same difference) are proportionally respired to a greater extent to provide energy. Such uncouplings of energy production and growth have been observed for bacteria limited by an essential nutrient or overly supplied with energy sources.[88] Support for this hypothesis is partially provided by the observed three-fold increases in the density of phenol degrading bacteria within 8 hr during the 24-hr incubation of the bio-oxidation assay reaction mixture (Table 61). The relationship between K_2^{bm} values for phenol, toluene, and aniline under continuous feed conditions are described in Fig. 50. The slope of this regression plot was interpreted as a second order rate coefficient, first order in both substrate and bacterial cell densities. This figure demonstrates several points: (1) that density of degradative populations was maintained at significantly different steady-state levels during the experiment, phenol>>aniline>>toluene, (2) the relative similarity in the rate of change of K_1^{bm} with increased densities of toluene and aniline degrading bacteria, which can be fit to a single (poorly correlated) regression line with a unifying slope K_2^{bm} of 4.8×10^{-9} μg substrate organisms^{-1} hr^{-1}; (3) the dissimilarity of K_2^{bm} value for phenol; and (4) and the overall variability K_1^{bm} values for individual degradative populations. It should be noted that in this series of experiments, toluene and aniline were added in the feedstock at a concentration of 200 and 100 mg/L; while phenol was added at a concentration of 1000 mg/L. The mixed liquor and effluent concentrations were consistently less than 1 mg/L because of the effects of the removal mechanisms.

A contributing explanation for the observed difference in K_2^{bm} values for phenol, and toluene and aniline, may include phenol toxicity[93,94] which is manifested in decreased growth rates beyond 100 mg/L. However, this

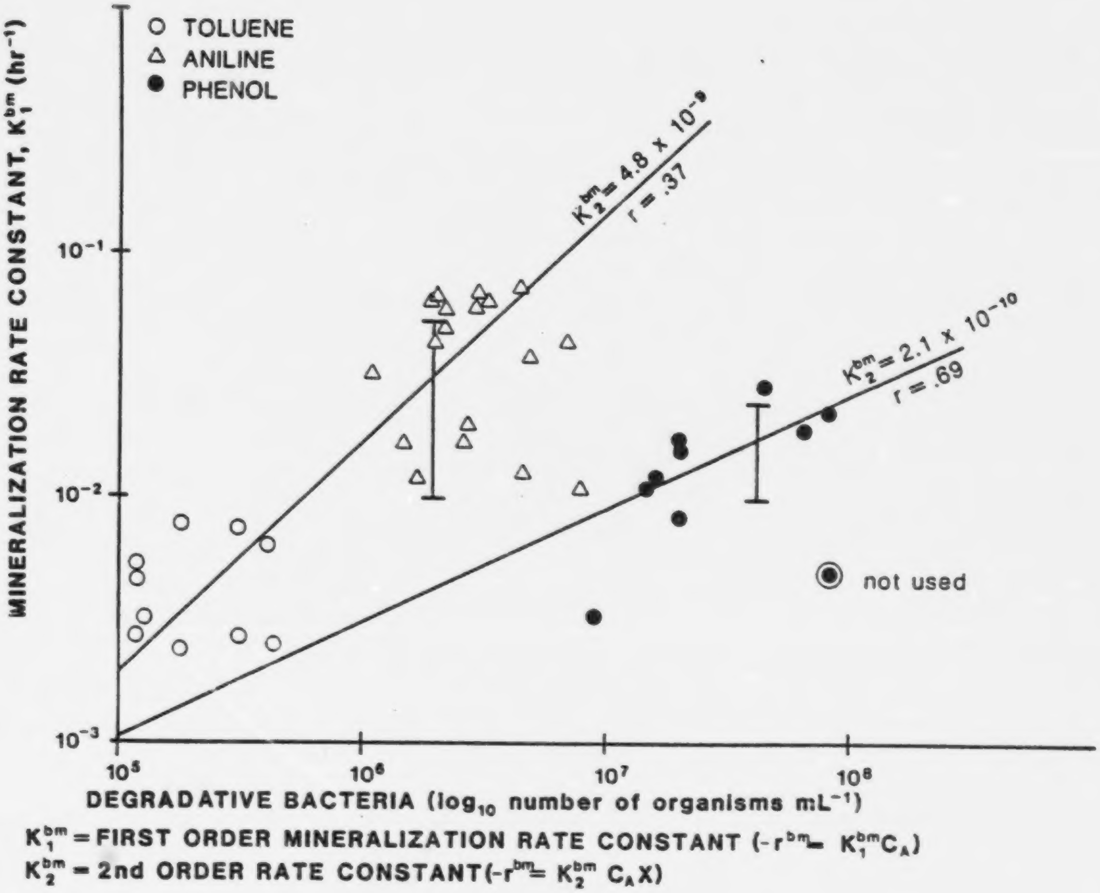

FIG. 50. RELATIONSHIP BETWEEN DEGRADATIVE BACTERIAL CELL DENSITIES AND SUBSTRATE MINERALIZATION KINETICS

effect can be discounted in batch assays which were conducted at 40 mg/L and in continuous studies in which the instantaneous phenol concentration was nearly undetectable.

Protozoan grazing was discounted as an explanation for observed differences in K_2^{bm} values for the three substrates. Active protozoa populations were qualitatively detected in all batch sludge samples used in bio-oxidation assays. Since it is likely that the degradative population for each substrate included members of the same species, it is difficult to envision protozoan feeding specificity that would demonstrate change as a result of alternative carbon sources and alternative biochemical pathways within the same organisms. Furthermore, mean K_2^{bm} values phenol mineralization approximately 2 to 2.5 X 10^{-10} mL org.$^{-1}$hr^{-1} as reported in this study are of the same magnitude as have been reported for pure culture studies of Pseudomonas putida grown on a chemically defined phenol medium.[92] In such studies, the K_2^{b} for phenol removal was reported at 7 X 10^{-9} (adjusted mL org.$^{-1}$hr^{-1} for volume differences). In comparison, for this study K_2^{b} for phenol removal can be calculated from data in Fig. 44C and this K_2^{b} value, 6.5 X 10^{-9} mL org.$^{-1}$hr^{-1}, represents a good approximation of the pure culture work.

An important yet unanswered question is the predictive nature and utility of bio-oxidation kinetic estimates derived from batch sub-sample assays of lab-scale continuous reactors. Comparative data from fill-and-draw reactor phenol removal rates and phenol removal rates in the mineralization assay system are available as was described in Fig. 44. These results indicated good correlation between the laboratory scale fill-and-draw reactor and the assay system employed. Consequently, mineralization data from the assay system should find utilization applications similar to those provided by batch reactors in predicting activated sludge performance for a given waste type.

On a comparative basis, phenol removal rates are an order of magnitude faster than mineralization of the substrate to CO_2 (under batch conditions). Since yield coefficients (g cells produced per g substrate utilized) reported for phenol may range from 0.5 to 0.6,[94] it is apparent that maximum quantities of substrate respired (CO_2 produced) in the assay system can account for 40 to 50% of the total substrate. (These are maximum values with phenol as the sole carbon source. This is not the case in this study.) The range of substrate respired in this study was 15 to 25% after maximum acclimation with the associated production of significant quantities of transient polar metabolites. Taking into account the rate of uptake of the substrate and conversion to intermediary metabolites, intracellular turnover of carbon and substrate complexity of the waste stream (feedstock), a tenfold difference in removal vs. mineralization rates is not surprising.

The complexity of feedstock must receive proper consideration in both fill-and-draw and continuous feed sludge acclimation, maintenance, and bio-oxidation kinetics. The foul condensate feedstock containing ca. 230 ppm methanol (~1/3 of the COD; see Section 5) is expected to be toxic to a proportion of the sludge community and will require significant acclimation.[95]

As the major carbon source the quantitative relationship between parent feedstock organic carbon and that added as test substrate will in part control total microbial density as well as quantity and biochemical capacity of the degradative population.

DISCUSSION

The fill-and-draw culture, biodegradation, and mineralization rate constants, calculated for phenol and aniline bio-oxidation data obtained in this study compare well with other literature reports on the fate of these compounds in both pure and mixed bacterial populations (Table 70). Phenol K_1^{bm} constants calculated from the pure culture data of Paris, et al.,[92] are nearly an order of magnitude greater than observed in this study. However, this approximate K_1^{bm} value was back calculated from K_2^{bm} using approximate cell density data[87] and is subject to some degree of speculation. However, as previously discussed, K_2^{bm} values for the two studies are essentially identical for phenol in fill-and-draw culture. Assuming first order kinetics, phenol mineralization rate constants as reported by Subba-Rao, et al.,[96] bracket the range of mean first order mineralization rate constants for phenol as observed in this study. The work of Subba-Rao, et al.,[96] indicates variations in predicted K_1^{bm} values for studies conducted at low versus high substrate concentrations suggesting lower K_1^{bm} at high substrate concentrations; i.e., greater than 100 mg/L. In the case of phenol, this effect may be related to toxicity. It is interesting to note that K_1^{bm} values for this study were more than an order of magnitude greater at 40 mg/L phenol when compared to this work at 100 mg/L phenol with observed K_1^{bm} values of $7.5 \times 10^{-4} hr^{-1}$. As previously demonstrated in this study, phenol toxicity may represent a significant inhibitory factor at concentrations approaching 100 mg/L (fill-and-draw) and can be responsible for the reported slower mineralization kinetics.[96]

Aniline mineralization rate constants (K_1^{bm}) observed in the present study were four-fold to an order of magnitude greater than reported in other studies, both mixed[96] and pure culture.[97] Data from a previous pure culture study[97] can be used to calculate aniline removal rate constants (K_1^{b}) that are in the approximate range of phenol K_1^{b} values reported here; with aniline K_2^{b} removal approximately four- to five-fold lower than those reported for phenol in this or a previous study.[92] It is interesting to note than aniline K_1^{b} values are in excess of an order of magnitude greater than K_1^{bm} values that follow the same pattern observed for phenol (Table 70).

These data comparisons support the conclusion that the kinetic responses observed in batch culture assays in this study fall within realistic limits observed by other investigations.

Comparative Bio-oxidation in Fill-and-Draw Acclimation Reactors

In general, fill-and-draw acclimation to phenol, toluene, and dichlorophenol achieved higher rates of bio-oxidation in stage 2 reactors during the initial 6 to 8 week acclimation response study period (Figs. 43, 45, and 46). However, the net increase in acclimation was greater for the stage 1

TABLE 70. COMPARATIVE PHENOL AND ANILINE BIODEGRADATION CONSTANTS AS REPORTED IN THIS AND RELATED INVESTIGATIONS

Substrate	Biodegradation rate constant				Reference
	K_1^b [a]	K_2^b [a]	K_1^{bm} [b]	K_2^{bm} [b]	
Phenol	$1.05 \times 10^{-1} - 1.37 \times 10^{-1}$	6.5×10^{-9}	$8.8 \times 10^{-3} - 2.9 \times 10^{-2}$	$2 \times 10^{-10} - 2.5 \times 10^{-10}$	This study
			$1.6 \times 10^{-4} - 5.5 \times 10^{-2}$ (7.5×10^{-4}) [c]	NA	32
	$\approx 7 \times 10^{-1}$ [d]	7×10^{-9}		NA	19
Toluene			$2.2 \times 10^{-3} - 4.5 \times 10^{-3}$	$1.1 \times 10^{-9} - 2.9 \times 10^{-9}$	This study
Aniline	NA	NA	4.3×10^{-2}	2.6×10^{-9}	This study
	NA	NA	$1.13 \times 10^{-2} - 5.75 \times 10^{-3}$	NA	32
	4.8×10^{-1} [d]	1.3×10^{-9} [d]	1.8×10^{-3} [d]	NA	33

[a] K_1^b, K_2^b; biodegradation 1st and 2nd order removal rate constants.

[b] K_1^{bm}, K_2^{bm}; bacterial mineralization 1st and 2nd order rate constants.

[c] Closest comparable substrate concentration is 100 mgL^{-1}.

[d] Calculated from available reported data.

NA, no available data for comparison.

reactors during the 6 to 8 week acclimation period and, on prolonged acclimation (15 weeks phenol and 20 weeks toluene), mineralization rates equaled or surpassed those achieved in the stage 2 reactors. From these results it can be concluded that acclimation is achieved faster in stage 2 reactors but maximum or sustained acclimation is attained in the higher BOD loading, stage 1 reactors. This effect is in part attributed to higher total bacterial cell and degradative bacterial cell densities maintained in the stage 1 reactors on prolonged acclimations (Figs. 41, 45, and 46). Reinforcing these observations are oxygen uptake data for phenol and catechol, which are demonstrably higher for both stage 1 toluene and phenol reactors than for companion stage 2 reactors (Tables 57, 58, 62, and 63). Oxygenase levels for phenol and toluene acclimated stage 1 reactors are also two- to three-fold greater than for the stage 2 reactors (Tables 56, 57, and 63).

A comparison of maximum attained first order mineralization rate constants (K_1^{bm}) for the three substrates examined during fill-and-draw acclimation is given in Table 71. In general, K_1^{bm} values are phenol>toluene>dichlorophenol, regardless of reactor stage. K_1^{bm} values of toluene and dichlorophenol are higher for stage 1 reactors as compared to stage 2 reactors; while the reverse is true for phenol. It should be noted that for the maximum stage 2 toluene, K_1^{bm} was attained using a flow through assay and this data point can be multiplied by a factor of three to accommodate substrate volatilization (this same conversion should be applied to all 1 to 10 week toluene acclimation data). In doing so, a K_1^{bm} (toluene) of 1.1 X 10^{-2} hr^{-1} for the stage 1 reactor is derived; identical to that stage 1 result. Such results further demonstrate the net similarity net acclimation among the reactors.

TABLE 71. COMPARATIVE SUMMARY OF MAXIMUM BIO-OXIDATION KINETICS IN FILL-AND-DRAW ACCLIMATION REACTORS

Substrate	Acclimation time	K_1^{bm} (hr^{-1}) Stage 1	K_1^{bm} (hr^{-1}) Stage 2
Phenol	6 wk		2.5 X 10^{-2}
	13 wk	2.0 X 10^{-2}	
Toluene	2 wk		3.7 X 10^{-3}
	20 wk	1.1 X 10^{-2}	
Dichlorophenol	5 wk	2.7 X 10^{-3}	4.9 X 10^{-3}

Major Limitations of Bio-oxidation, Population, and Enzyme Measurements

The results of this study indicate that potential may exist for predicting bio-oxidation mineralization kinetics based on observed population and enzyme levels. A major limitation to overcome in enzyme analysis is sensitivity of existing enzyme assays. The majority of results of enzyme activity in this study were borderline sensitivity. Even though total cell densities were maintained at relatively high levels, i.e., $>10^9$ org mL^{-1}, total degradative cells for the individual substrates accounted for less than 0.1% of the total population. Consequently, associated specific enzyme levels would be expected to be low. As a result, only broad specificity enzymes, catechol oxygenases and parent substrate oxygenases, could be detected in this study. The obvious modification is to scale up enzyme assays to use 10- to 100-fold larger sample sizes. Confounding factors are multiple enzyme pathways, induction, and enzyme repression pathways, induction, and enzyme repression effects reported by other investigations that make specific enzyme selection difficult.[93,98,99] Unless a waste is dominated by one carbon source (as in the continuous feed phenol studies) broad spectrum enzyme activity is likely to provide less ambiguous information than specific enzymes. This approach may prove less useful than other assays of metabolic activity, such as mineralization or oxygen uptake.

A possible indication of foul condensate feedstock inhibition of phenol oxidation was observed by comparing phenol mineralization of washed sludge culture to whole sludge cultures. As indicated by Table 72, increased phenol K_1^{bm} values were demonstrated in washed sludge cultures for both stage 1 and 2 fill-and-draw acclimation reactors. The net increase in stage 1 K_1^{bm} value was 21% as compared to 17% for the stage 2 K_1^{bm} and this difference may be indicative of a lower level interference in the stage 2 feedstock. Janke, et al.,[69] observed similar repression of phenol biodegradation by the aromatic compounds, aniline and benzoate, as well as catechol ring cleavage products.

TABLE 72. COMPARISON OF PHENOL MINERALIZATION BY WASHED AND WHOLE SLUDGE SAMPLES

Sludge sample	Substrate respired: Cumulative[a] (μg mL^{-1}) replicate	mean	Rate (μg mL^{-1} hr^{-1})	K_1^{bm}
Reactor 1				
Whole sludge	3.76 1.55	2.65	1.1×10^{-1}	2.75×10^{-3}
Washed sludge[b]	4.15 2.58	3.37	1.4×10^{-1}	3.5×10^{-3}
Reactor 2				
Whole sludge	5.14 3.2	4.17	1.7×10^{-1}	4.3×10^{-3}
Washed sludge	5.3 4.8	5.05	2.1×10^{-1}	5.2×10^{-3}

[a] 24 hr period.

[b] An equal volume of sludge washed with phosphate buffered saline (PBS) and resuspended in PBS at the same concentration as whole sludge. Phenol assay concentration, 40 mg/L.

The enumeration of low levels of degradative cells for specific substrates is associated with low enzyme levels. This problem is most clearly demonstrated by the failure to demonstrate specific 2,4-dichlorophenol degrading populations. This may have been the result of toxicity of the enumeration medium or co-oxidative-consortium population responsible for degradation of the substrate. In routine practice it is assumed that 10% or less of the viable bacterial cells can be recovered, even on nutritionally complex media. In the case of chemically defined media with poor growth substrates, recovery of viable populations may be more difficult due to factors such as toxicity, auxotrophy (growth factor requiring mutants), substrate transport, physical diffusion, etc. Developing methods using specific gene probes to detect cells harboring specific degradative genes are likely to improve the sensitivity and accuracy of estimating degradative cells densities in mixed populations (Sayler, et al., in preparation).

The bio-oxidation assay has demonstrable utility in assessing rates of pollutant mineralization (terminal oxidation fate) and the response of mixed pollutants to substrate acclimation. A major problem associated with mineralization assay is encountered when examining volatile or insoluble substrates. Such substrates require extra care in assuring that captured $^{14}CO_2$ is an authentic and not volatile substrate. This can be accomplished by absorption of volatile substrates in the exit gas of the assay system and by barium salt precipitation of the CO_2 as $BaCO_3$. Loss of volatile and insoluble substrates to reaction vessel components must also be monitored to obtain an acceptable mass balance. A further problem is the delivery of the substrate to the reaction vessel. This problem was most serious with toluene as a substrate. Toluene had to be added in methanolic solution or in the foul condensate feed stock to assure uniform distribution in the reaction mixture and to reduce substrate volatilization.

Problems in comparing bio-oxidation kinetics in fill-and-draw systems measured with batch assays to continuous culture studies represent a limitation on data interpretation and extrapolation. Under fill-and-draw culture conditions, a steady-state cannot be achieved. However, under continuous culture condition multiple steady states can be maintained for different populations when continuous selective pressure is applied to the entire mixed population.[93] Unbalanced growth which may be seen as near complete mineralization of a substrate with little incorporation in cell mass has been observed for natural mixed population under batch conditions.[96] Such activities and growth kinetics may be expected in mixed continuous populations, depending on the feedstock composition and growth rates of component populations. This issue is further discussed in Section 10.

SECTION 10

LABORATORY SCALE ACTIVATED SLUDGE STUDY

SUMMARY

The purpose of this study was to quantify the removal mechanisms of four organic substrates in continuous flow, completely mixed activated sludge units. Two 11-liter lab-scale activated sludge (LAS) units (reactors 1 and 2) were operated at mean cell residence times of approximately 5 and 10 days. Data were to be used to test the predictive fate methods which had previously been developed for stripping, sorption, and bio-oxidation.

Material balances around the activated sludge units revealed that the fate of phenol and aniline are almost identical, with >99.8% of the parent compound bio-oxidized and with stripping losses below detectable limits. Approximately 56% of the ^{14}C in the feed ^{14}C phenol was recovered as ^{14}C in $^{14}CO_2$ for both compounds. Removal of these compounds from activated sludge units by sorption is insignificant on a material balance basis, with average compound loadings of <84 μg/g MLSS and 28 μg/g MLSS determined for phenol and aniline, respectively.

Biological oxidation was also determined to be the major removal mechanism for the toluene, the most volatile of the test compounds. Approximately 67 to 70% of the feed toluene ^{14}C was recovered as $^{14}CO_2$ and only 5 to 6% of the ^{14}C was recovered as soluble metabolites. The ability of the activated sludge systems to biologically degrade toluene was found to be very sensitive to deviations from steady-state operating conditions (flow fluctuations, etc.). Grab samples of mixed liquor indicated variations in the concentration of toluene in the aqueous phase to range from <0.05 mg/L to 4.7 mg/L.

Stripping was determined to be a secondary removal mechanism, with approximately 12% of the feed toluene stripped from both reactors. However, the mass stripped from solution was highly variable from day to day and was shown to be highly correlated to the concentration of toluene in the aqueous phase. During periods of ideal steady-state operation, toluene concentrations in the vent gas were below the detectable limit of 0.75 ppm_v, indicating that less than 0.4% of the feed toluene was stripped from solution.

Pentachlorophenol was found to be extremely resistant to biological oxidation and stripping. Sorption onto sludge was determined to be the major

removal mechanism, with both ^{14}C and pentachlorophenol analyses indicating that 6 to 8% of the feed pentachlorophenol was removed by sorption to waste solids. The average pentachlorophenol loading on biological solids was determined to be 1967 ug/g MLSS and 1801 ug/g MLSS for reactors 1 and 2, respectively.

The predictive fate method which was developed for stripping was determined to be accurate in predicting the concentration of test compounds in the vent gas as a function of the measured concentration in the aqueous phase, the Henry's law constant, and the relative air flow/liquid volume ratio. Predicted vent gas concentrations of toluene were within ±50% of observed values for approximately 70% of the observations. The remaining observations had either or both vent gas and/or mixed liquor concentrations below the detectable limit. These comparisons were made with daily average data, and when vapor and liquid concentration data for samples taken at the same time were analyzed, the precision of the predictor improved.

Average loadings of aniline, phenol, and pentachlorophenol onto biological solids were found to be related to the octanol-water partition coefficient (K_{OW}). However, the proposed predictive equation for relating equilibrium loading to the equilibrium aqueous phase concentration was found to predict loadings which were factors of 20 and 360 lower than observed for phenol and aniline, respectively. Conversely, predicted pentachlorophenol loadings were an order of magnitude higher than those observed. However, when the K_{OW} of pentachlorophenate was used, the agreement between predicted and measured loadings improved markedly.

Poor agreement between observed and predicted loadings for the compounds with low octanol-water partition coefficients may be partially related to analytical variability in extracting extremely small amounts of sorbed material from the biomass and in accounting for the residual soluble compound in the extracellular water of the sludge sample. Phenol and aniline were also shown to exhibit high biodegradation rates. Active transport and storage of substrate within the cell may be a significant removal mechanism for readily degradable hydrophobic substrates.

Pentachlorophenol sorption was shown to be described by a Langmuir isotherm model in which a saturation loading is reached where loading does not increase at increasing equilibrium aqueous phase concentrations. Pentachlorophenol was found to be non-biodegradable under the LAS test conditions, yielding effluent concentrations above the concentration required for saturation of the biomass relative to pentachlorophenol loading.

However, Langmuir isotherm parameters developed from data derived from both batch and LAS studies indicate excellent agreement between the proposed model and results reported by other researchers for compounds with K_{OW} values between 100 and 10,000 and in the range where the relationship between equilibrium aqueous phase concentrations and loadings were linear.

Mineralization rates for phenol and toluene in fill-and-draw reactors were significantly lower than rates obtained in the continuous systems while aniline exhibited higher rates of mineralization in the batch tests than observed in the continuous systems. Similar results were obtained for phenol based upon disappearance of the parent compounds. Several factors may contribute to the differences in mineralization rates observed in the batch tests and the continuous reactors. Initial concentrations of phenol and toluene used in the batch tests may have been inhibitory to the biomass, yielding low rates of mineralization for these compounds. Test compound mass transfer limitations and oxygen transfer limitations might also lead to apparently low mineralization rates. However, tests with aniline would tend to contradict this last hypothesis.

The lower aniline mineralization rates observed in the continuous reactors may have been due to the rate at which aniline was supplied to the systems. Both continuous systems reached an equilibrium state in which approximately 56% of the ^{14}C in the radiolabeled aniline was mineralized to $^{14}CO_2$. Batch test results indicate that increased rates of aniline mineralization could have been obtained at higher aniline feed concentrations in the continuous systems.

Presently, no acceptable explanations exist for the wide variance, two to four orders of magnitude, between disappearance rates in the fill-and-draw and continuous reactors. A basic difference in microbial kinetics between steady- and unsteady-state systems for aromatic biotransformation is possible.

BACKGROUND

Overview

There were two primary objectives of this study. The first was to develop experimental equipment and methods to quantify the removal mechanisms of organic compounds in activated sludge processes. Removal mechanisms were to be quantified both in terms of the parent compound and a material balance based on the ultimate fate of carbon in the parent compound (mineralization to CO_2, conversion to metabolite, etc.). The second objective was to attempt to verify the predictive fate methods which previously had been developed for stripping, sorption, and bio-oxidation.

A number of studies have focused on parts of the overall problem. Data from several investigators have shown excellent correlations between equilibrium loading of organic compounds onto biological solids and the octanol-water partition coefficient.[6,18,34] Previous experimental work also suggests that stripping rate constants are correlated with Henry's law constants and the oxygen mass transfer coefficient.[6,34] The most difficult aspects of predicting fates of organic chemicals appear to be in developing reliable estimates of biological oxidation rates. Design equations based on the Monod equation have been applied successfully to systems with a single organic carbon source or for which the substrate is measured by a nonspecific parameter (TOC, COD, BOD_5).

Design equations based upon the Monod model assume that the organic substrate is the growth-limiting nutrient, the rate of substrate disappearance approaches first order at low substrate concentrations and zero order at high substrate concentrations, and that the growth yield coefficient is a constant and may be derived in terms of the growth-limiting carbon source.

Application of equations similar to the Monod equation to the prediction of the fate of a single substrate in a wastewater with heterogeneous biological populations and multiple carbon sources obviously presents a multitude of potential problems. Work described in Section 9 of this report described experimental work aimed at addressing two of these problems:

- The effect of alternative carbon sources (via the two-stage reactor system), and
- The investigation of fraction of the total biological population capable of degrading a specific compound

The second of these issues has been addressed in the LAS studies in conjunction with work at the University of Tennessee by providing sludge cultures from continuous reactors for estimation of the fraction of the biological population which actively degrades a test compound.

Related Studies --

Since 1980, the results of a number of studies have been published in which the interrelationships of the bio-oxidation/stripping/sorption mechanisms were investigated. Some of these studies have investigated all three mechanisms while others have concentrated on only two. Several of these studies have focused on one or more of the same compounds which were used in the LAS study. The most important studies will be referenced and discussed here.

The most comprehensive study of the fate of organic chemicals in full-scale biological treatment plants is a U.S. EPA Effluent Guideline Division study of 40 publicly owned treatment works which was initiated in 1978.[100] The final report for this project also includes data for 10 supplemental plants which were sampled under a parallel project. Plants chosen for the study were selected to represent a broad range of size, type, and amount of industrial contribution, operating efficiency, geographical distribution, and treatment processes. All of the plants provided a minimum of secondary treatment with 38 of the plants employing the activated sludge process as part of the process train. At most of the plants, a minimum of 6 days of 24-hour sampling of influent, effluent, and sludge streams was completed. Each sample was analyzed for conventional, selected non-conventional, and priority pollutants.

Toluene, phenol, and pentachlorophenol were among the 20 most commonly detected organic compounds in the influents to the 40 POTW, being detected in 96, 79, and 29% of the influent samples, respectively. These compounds were also among the 15 most commonly detected organic compounds in the POTW effluents, with toluene detected in 53% of the samples, phenol in 29% of the samples, and pentachlorophenol in 21% of the samples. Toluene, phenol, and pentachlorophenol were among the 25 most commonly detected organic compounds in the raw sludges with 94, 50, and 14%, respectively, of the samples having detectable quantities of these compounds. Raw sludges were defined as all unthickened or thickened primary, secondary, or combined sludges.

Pellazari[18] conducted a pilot study to determine the concentrations of volatile priority pollutants in the off-gas from the aeration basin and in the activated sludge and the raw wastewater at the Northside Treatment plant in Durham, North Carolina.[18] Thirteen volatile compounds were detected in the off-gas from the aeration basin. In general, the concentrations in the off-gas decreased by a factor of 2 to 3 from the front of the aeration basin to the end of the aeration basin. However, the concentrations of priority pollutants in the aqueous phase did not exhibit a gradient across the aeration basin.

Experiments were also conducted with ^{14}C-labeled chlorobenzene, toluene, benzene, chloroform, trichloroethylene, carbon tetrachloride, and bromobenzene to determine the extent of sorption by activated sludge solids. Initial concentration of the test compounds in the range of 1 to 100 μg/L in methanol was spiked into 10 mL of activated sludge in a 50-mL centrifuge tube. Samples were mixed with a magnetic stirrer for 18 hours at 4°C. After equilibrium, solids were removed by centrifugation and aliquots of the supernatant were analyzed for radioactivity. An aliquot of the solids was also measured for radioactivity.

The Freundlich sorption isotherm relationship was used to describe the equilibrium loadings of each compound onto activated sludge solids. The Freundlich adsorption isotherm has the form:

$$C_s = K\, C_{oe}^{\,n} \qquad \text{Eqn. 58}$$

where:

C_s = equilibrium concentration in the sludge (μg compound/g total suspended solids),

C_{oe}= concentration in the aqueous phase (μg/L),

K = sorption capacity coefficient, and

n = sorption intensity coefficient.

Sorption studies conducted at different time periods indicated large variations in values for both K and n. Significant sorption capacities were noted for activated sludge solids; however, the sorption capacity for the same chemicals to solids in the raw wastewater was deemed negligible. A summary of the Freundlich isotherm parameters which were determined for activated sludge are presented in Table 73.

TABLE 73. VARIATION OF FREUNDLICH ADSORPTION ISOTHERM PARAMETERS FOR SELECTED PRIORITY POLLUTANTS IN ACTIVATED SLUDGE - MEAN ± STANDARD DEVIATION[a]

Compound	Freundlich n	Freundlich K
Chloroform	1.022 ± 0.218 (21)	2.04 ± 1.91 (93)
Carbon tetrachloride	1.037 ± 0.211 (20)	13.8 ± 1.74 (13)
Chlorobenzene	0.934 ± 0.283 (30)	3.54 ± 1.95 (55)
Toluene	0.935 ± 0.258 (28)	4.36 ± 1.95 (45)
Benzene	1.102 ± 0.466 (42)	2.88 ± 1.99 (69)
Trichloroethylene	1.073 ± 0.300 (28)	4.57 ± 3.02 (66)

[a] See ref. 18.

Studies were also conducted to determine the time required to achieve equilibrium between the aqueous phase and activated sludge solids for ^{14}C-labeled bromobenzene and chlorobenzene. Equilibration times of 30 min and 18 hr were used. The partitioning in the two phases was virtually identical for both chemicals at both equilibrium times. These results indicate that sorption to the solid phase occurs rapidly and an apparent equilibrium is reached in 30 min or less.

Lurker, et al.,[101] conducted field studies to evaluate aqueous, sludge, and off-gas concentrations of several chlorinated compounds at the North Wastewater Treatment Plant, Memphis, Tennessee. Compounds which were studied include hexachlorocyclopentadiene, (HEX), hexachlorocyclopentadiene bicycloheptene (Hex-BCH), heptachlorobicycloheptene (HCBCH), chlordene, carbon tetrachloride, chloroform, and tetrachloroethylene. Wastewater samples were collected at the wet well, grit chamber, contact basin, stabilization basin, digester basin, and the final effluent. Air sample locations included the grit chamber and each end and center of the aeration basins.

The concentrations of the Hex-related compounds (Hex-BCH, HCBCH, chlordene) tended to increase as the wastewater flowed through the plant from the contact basins to the stabilization basins to the digestors. Wastewater samples were extracted without separating the biological solids from the aqueous phase; therefore, no quantitative estimates can be made of the true compound loadings on the biological solids. However, the authors conclude that these results indicate a significant sorption of these compounds to the suspended solids.

Data collected over a 30-day period showed that concentrations of these three compounds in the digested sludge were approximately 13 times higher than in the plant influent. Conversely, the more water-soluble compounds (carbon tetrachloride, trichloroethylene, and chloroform) showed a marked reduction in each unit process of the treatment system. These compounds were shown to be predominantly stripped from solution, with the highest airborne concentrations measured at the grit chamber weir.

Petrasek, et al.,[34] investigated the fates of 16 volatile compounds in a 35-gpm conventional plug flow activated sludge pilot plant located at the U.S. EPA Test and Evaluation (T&E) facility at Cincinnati, Ohio. Average operating conditions of the pilot plant were as follows:

- 2900 mg/L MLSS
- 5.9 day solids retention time
- 2.11 L/sec influent flow
- 56.6 L/sec airflow

Degritted and comminuted raw wastewater was pumped from the Cincinnati Mill Creek Wastewater Treatment plant to a head tank, and the feed wastewater was continuously spiked with a methanol solution containing the 16 organic compounds. During the 12-month study, a total of 20 sets of samples were collected for volatile organics analysis. Off-gas from the aeration basins was sampled with Tenax traps, and grab samples of the effluent samples were collected in 40-mL glass vials with Teflon®-sealed screw caps.

The mean concentrations observed in the experimental treatment sequence are shown in Table 74, and a summary of the material balance around the experimental system is shown in Table 75. Volatilization from the primary clarifier and stripping from the aeration basin are shown to be major removal mechanisms while extremely low levels of the test compounds were recovered from the secondary sludge. Although the author states that "partitioning in the primary sludge was not a significant removal mechanism," data in Table 74 indicate that >5% of the toluene, ethylbenzene, tetrachloroethylene, and tetrachloroethane in the influent partitions in the primary sludge.

Preliminary experiments[34] on sorption kinetics were conducted by spiking the test compound into 1.5-L samples of mixed liquor in a 2-L beaker with a floating Teflon cover. The sample was mixed with a magnetic stirrer and samples were withdrawn after 5, 10, 15, 30, and 60 min. The samples were immediately centrifuged and the aqueous phase organic concentrations determined by GCMS. The concentration vs. time curves indicated that the sorption was first order, and the sorption coefficients were computed accordingly. The sorption rate constants were found to be highly correlated to the octanol-water partition coefficient (K_{ow}), with a correlation coefficient of 0.95 reported for one study with eight compounds. First order sorption rate constants for seven of the eight compounds were in the range of 3 to 8 hr^{-1} for K_{ow} values ranging from approximately 100 to 500. An equation relating the sorption rate constant to the octanol-water partition coefficient was presented as follows:

$$K_a^{sp} = 0.007\ K_{ow} + 3.02 \qquad \text{Eqn. 59}$$

where:

K_a^{sp} = first order sorption rate constant (hr^{-1}) and

TABLE 74. MEAN CONCENTRATIONS OBSERVED IN THE EXPERIMENTAL TREATMENT SEQUENCE[a]

	Influent (µg/L)	Primary effluent (µg/L)	Primary sludge (µg/L)	Return activated sludge (µg/L)	Activated sludge effluent (µg/L)	Aeration basin off-gas (ng/L)	Removal by primary clarifier (percent)	Removal by treatment sequence
Methylene chloride	118	89	<143	<21	<4.0		25	>97
1,1-Dichloroethane	79	34	<40	<1	<0.2	425	57	>99
Chloroform	137	143	<208	<7	3.6	838	0	>97
Carbon tetrachloride	60	32	<14	<1	<0.2	322	47	>99
1,2-Dichloropropane	309	295	<461	<1	<6.0	1628	5	>98
Trichloroethylene	107	68	389	<1	<1.5	609	36	>99
1,1,2-Trichloroethane	133	155	<219	31	28.0	663	0	80
Dibromochloromethane	58	49	<10	<2	<7.0	1291	16	>88
Benzene	73	61	121	<1	<0.2	225	16	>99
1,1,1-Trichloroethane	132	96	<220	<1	<0.3	1022	27	>99
Bromodichloromethane	89	64	<25	<1	<0.2	1155	28	>99
Chlorobenzene	197	163	953	<5	<1.3	149	17	>99
Tetrachloroethylene and Tetrachloroethane	252	263	2033	25	16.0	1227	0	94
Toluene	255	198	974	<2	<0.6	672	22	>99
Ethylbenzene	82	69	766	<1	<0.2	243	16	>99

[a] See Ref. 34.

TABLE 75. SUMMARY OF MASS BALANCES ON THE EXPERIMENTAL SYSTEM[a]

	Percent of each compound found at indicated sample location			
Compound	Primary[b] sludge	Activated[c] sludge effluent	Waste[c] activated sludge	Aeration[c] basin off-gas
Methylene chloride	1.4	<0.5	<0.3	
1,1-Dichloroethene	<0.7	<0.1	<0.1	72
Chloroform	2.0	<0.2	<0.1	34
Carbon tetrachloride	<0.3	<0.1	<0.1	59
1,2-Dichloropropane	<2.0	<0.2	<0.1	32
Trichloroethylene	4.7	<0.3	<0.1	41
1,1,2-Trichloroethane	2.1	<1.8	0.4	25
Dibromochloromethane	<0.2	<1.4	<0.1	>100
Benzene	2.3	<0.1	<0.1	15
1,1,1-Trichloroethane	2.1	<0.1	<0.1	62
Bromodichloromethane	<0.4	<0.1	<0.1	>100
Chlorobenzene	<6.6	<0.1	<0.1	5
Tetrachloroethylene and tetrachloroethane	10.7	0.6	0.2	27
Toluene	5.1	<0.1	<0.1	20
Ethylbenzene	12.3	<0.3	<0.1	21

[a] See Ref. 34.

[b] Based on influent concentrations.

[c] Based on primary effluent concentrations.

K_{ow} = octanol water partition coefficient.

No procedures were reported to eliminate or correct for biological oxidation during the sorption studies.

Weber[35] has conducted similar rate studies in glass 2.5-L completely mixed batch reactors stirred with magnetic stirring bars. Mixed liquor was taken from the aeration section of a biological reactor and added to the 2.5-L batch reactor. The solution was saturated with dissolved oxygen, spiked with the five test compounds at concentrations between 60 and 70 µg/L, and sealed with a lid to minimize volatilization losses. A dissolved oxygen probe was inserted through a hole in the lid to measure the concentration of dissolved oxygen. Samples were taken at 2 to 3 min intervals through a small hole in the reactor lid with a 10-mL syringe, filtered, and stored in capped sample bottles at 4°C. A given rate study was terminated when the dissolved oxygen dropped to 1.0 mg/L. Removal was considered to be due to biodegradation, and first order bio-oxidation rate constants have been determined for each compound. A second study conducted using bacteria inactivated with $AgNO_3$ showed no decrease in the aqueous phase concentration in any of the compounds beyond a small amount attributed to volatilization. Studies were also conducted to evaluate the biodegradation rate of each compound when added separately to the batch reactor and to evaluate the biodegradation rates when all five compounds were spiked at initial concentrations between 270 and 290 µg/L. Biodegradation disappearance rate constants measured for the single compound study were a factor of approximately 2 to 3 higher than measured for the same compound when added in a mixture of five compounds. Rate constants were found to be depressed by a factor of approximately 3 to 7 when all five compounds were added at concentrations of 270 to 315 µg/L as compared to rates measured at initial concentrations of 60 to 70 µg/L. Results of these studies are summarized in Table 76.

TABLE 76. FIRST ORDER BIODEGRADATION DISAPPEARANCE RATE CONSTANTS[a]

	5 Compound study						
		May 21	May 28	5 Compound study		Single compound study	
Compound	C_{ao} (µg/L)	K_1[b] (hr^{-1})	K_1[b] (hr^{-1})	C_{ao} (µg/L)	K_1[b] (hr^{-1})	C_{ao} (µg/L)	K_1[b] (hr^{-1})
Benzene	60-70	2.70	2.58	282	0.72	60-70	4.86
Toluene	60-70	3.84	3.18	278	0.72	60-70	7.08
Chlorobenzene	60-70	0.84	0.90	315	0.30	60-70	3.19
Ethylbenzene	60-70	5.22	3.30	270	0.72		
o-Xylene	60-70	ND	5.58	286	1.02		

ND = Not determined.

[a] See ref. 35.

Matter-Muller, et al.,[6] evaluated the treatment mechanisms for n-heptadecane, pristane, 1,2,3-trimethylbenzene, and 1,4-dichlorobenzene in two parallel activated sludge pilot plants during two different study periods. Operating conditions for these two plants are given in Table 77. Wastewater flow to the pilot plants consisted of domestic wastewater which had been screened and pre-precipitated with $FeCl_3$. The test compounds were indigenously present in the wastewater at concentrations ranging from 1.0 to 5.0 μg/L.

TABLE 77. OPERATING CONDITIONS FOR SECONDARY TREATMENT PILOT PLANTS[a]

Plant No.	Period No.	Flow (m^3/day)	Tank volume (m^3)	Sludge age (days)	Sludge production (kg TS/day)
3	1	91.2	11.3	3.1	8.5
	2	110.4	11.7	3.1	10.5
4	1	55.2	15.0	7.6	4.9
	2	50.4	11.7	7.6	5.1

[a] See Ref. 6.

The concentration of the test compounds was measured in the primary effluent, the secondary effluent, and sorbed to the activated sludge. Sorption to waste solids was shown to be the primary removal mechanism for n-heptadecane and pristane while volatilization to the atmosphere was postulated to be the primary removal mechanism for 1,2,3-trimethylbenzene and 1,4-trichlorobenzene. The results of the material balances are shown in Table 78.

The test compounds that were studied were relatively non-biodegradable, therefore bio-oxidation was not considered to be a significant removal mechanism. The mass flux of materials to the atmosphere shown in Table 78 was estimated using a technique in which the mass transfer rate for the test compounds was related to the oxygen mass transfer rate. The ratio of the mass transfer rate coefficient for the volatile test compounds to the mass transfer rate coefficient for oxygen was shown to be a constant value for a given compound and was independent of temperature, degree of turbulence, and other conditions in the aqueous phase.

A series of studies have been conducted at Oklahoma State University to evaluate the removal mechanisms for toxic priority pollutants.[4,19,20] Three-L activated sludge units were operated at mean cell residence times of 2, 4, and 6 days, hydraulic residence times of 8 hr, and an air flow rate of 2 to 3 L/min. Wastewater feed to the system consisted of a base mix consisting of ethylene glycol, ethyl alcohol, acetic acid, glutamic acid, glucose, phenol, inorganic nutrients and a specific test compound.

TABLE 78. MASS BALANCE FOR TEST COMPOUNDS AS A PERCENT OF INFLUENT MASS FLOW[a]

Compound	Plant No.	Period No.	Waste solids	Soluble in secondary effluent	Vent gas
n-Heptane	3	1	81	4	2
		2	95	4	2
	4	1	65	3	22
		2	27	4	3
Pristane	3	1	61	<1	1
		2	80	<1	1
	4	1	100	<1	7
		2	100	<1	1
1,2,3-Trimethylbenzene	3	1	0	9	62
		2	<1	4	24
	4	1	0	11	>100
		2	<1	6	86
1,4-Dichlorobenzene	3	1	0	ND	ND
		2	7	10	57
	4	1	0	ND	ND
		2	0	20	>100

ND = not detected.
[a] See Ref. 6.

Initial studies were conducted to compare stripping in activated sludge systems with stripping in non-biological systems. The non-biological systems were operated at conditions similar to those for the activated sludge studies except that no biomass was present in the reactors.

The results of this study are shown in Table 79. A number of compounds, including methylene chloride, benzene, ethyl acetate, and 1,2-dichlorobenzene were highly stripped in the non-biological systems and only slightly stripped in the activated sludge system. These results indicate that the combined rates of sorption and bio-oxidation are significantly higher than the stripping rate for these four compounds.

Results of a later study in which the removal mechanisms were quantified for 15 priority pollutants are shown in Table 80. Overall removal for all compounds was extremely high. Sorption onto biological solids was shown to be negligible for all compounds studied, as would be expected since the octanol-water partition coefficients for all test compounds are relatively low. Data reported in Table 80 correlate well with biodegradation data reported by Tabak, et al.[33] Compounds reported to be highly stripped in the

TABLE 79. MATERIAL BALANCES FOR ACTIVATED SYSTEMS AND NON-BIOLOGICAL SYSTEMS[a]

	Activated sludge system						Non-biological system
Compound	Sludge age (days)	Influent (mg/min)	Effluent (mg/min)	Off-air (mg/min)	Overall removal efficiency	Percent stripped	Percent stripped
1,2-Dichloropropane	2	0.925	0.006	0.813	99.4	87.9	98.8
	4	0.922	0.008	0.813	99.1	88.2	
	6	0.914	0.009	0.813	99.0	88.9	
Methylene chloride	2	0.992	0.005	0.046	99.5	4.6	99.4
	4	1.149	0.006	0.037	99.5	3.2	
	6	1.159	0.006	0.079	99.5	6.8	
Benzene	2	0.941	0.001	0.157	99.9	16.7	99.3
	4	1.151	0.0005	0.157	99.9	13.6	
	6	1.008	0	0.159	100	15.8	
Ethyl acetate	2	0.896	0.008	0.119	99.1	13.2	81.9
	4	0.896	0.004	0.286	99.6	31.9	
	6	0.992	0.0003	0.064	99.9	6.5	
1,2-Dichloroethane	2	1.317	0.0188	1.331	98.6	101.1	96.1
	4	1.320	0.0126	1.612	99.0	122.1	
	6	1.376	0.0230	2.230	98.3	162.0	
Phenol	2	1.085	0.00006	0	>99.9	0	1.9
	4	1.058	0.00006	0	>99.9	0	
	6	1.031	0.00006	0	>99.9	0	
1,2-Dichlorobenzene	2	0.602	0	0.125	>99.9	20.8	84.7
	4	0.607	0	0.180	>99.9	29.7	
	6	0.605	0	0.131	>99.9	21.7	

[a] See Refs. 4, 19, 20.

TABLE 80. MATERIAL BALANCE FOR PRIORITY POLLUTANTS IN COMPLETE-MIX ACTIVATED SLUDGE[a]

Compound	Influent (mg/L)	Effluent (mg/L)	Percent overall removal	Percent removed by		
				Stripping	Sorption	Biodegradation
Tetrachloroethane	201	11.0	94.5	100	0	0
Nitrobenzene	100	2.2	97.8	0	0	100
2,4-Dichlorophenol	75	3.6	95.2	0	0	100
Acrelein	62	<0.05	99.9	0	0	100
Acrylonitrile	152	<0.05	>99.9	0	0	100
1,2-Dichloropropane	182	1.81	99.0	89	b	b
Methylene chloride	180	0.51	99.7	7	0	93
Ethyl acetate	167	0.21	99.9	7	0	93
Benzene	153	0.04	99.9	16	0	84
1,2-Dichlorobenzene	258	3.70	98.6	99	1	0
Phenol	173	<0.01	>99.9	0	0	100
1,2-Dichlorobenzene	83	<0.05	>99.9	22	0	78
1,1,1-Trichloroethane	118	1.60	98.6	100	0	0
1,3-Dichlorobenzene	69	0.36	99.5	c	c	c
2,4-Dinitrophenol	96	<0.66	>99.3	0	1	99

[a] See Refs. 4, 6, and 19.

[b] No biodegradation observed.

[c] No 1,3-dichlorobenzene found in off-gases; however, two unidentified gases observed.

Oklahoma State study, tetrachloroethane, 1,2-dichloropropane, 1,2,-dichloroethane, and 1,1,1-trichloroethane, were found by Tabak to be relatively resistant to biodegradation when compared to the other priority pollutants.

Studies were also conducted in 2.5-L batch reactors with phenol, acrylonitrile, and 2,4-dichlorophenol. Batch reactors were operated at a mean cell residence time of 3 days. Air flow rate to the batch reactors was maintained at 2.0 L/min. Effluent samples were taken at 1, 2, 3, 4, 5, 8, 10, and 20 hr. Results of the batch studies are presented in Table 81. Overall removal of phenol and acrylonitrile was less than that achieved in the continuous reactors. Stripping was shown to account for 25% of the overall removal in the batch reactor while none of the acrylonitrile was stripped from the continuous system. Poor removal of 2,4-dichlorophenol in the batch reactor was attributed to inhibition of bio-oxidation at the concentration used in the batch test.

MATERIALS AND METHODS

Process Equipment

Lab-scale activated sludge (LAS) studies were conducted in two continuous flow, completely mixed 11-L reactors. A flow sheet of a LAS system is shown in Fig 51. Materials of construction are primarily glass, stainless steel, and Teflon. Individual components of the system are discussed in detail below.

Air Supply System --

Compressed air was supplied from the building air system. A backup air cylinder was automatically activated if pressure loss occurred in the building air supply. Air pressure was regulated and indicated by a pressure regulator and pressure gauge. Supply air was passed through a carbon column constructed of 6.35 mm (2.5 in.) OD X 50.8 mm (20 in.) PVC pipe with end caps. The carbon column was packed with Barneby-Cheney AC2143 activated carbon. The air supply was then passed through a water scrubber and a demister packed with glass wool. Both the water scrubber and the demister were constructed of 10.2 mm (4-in.) OD X 45.7 (18 in.) clear PVC. The air flow to the reactor was measured and controlled by a rotameter equipped with a micrometer valve.

Feed System --

The feed system consisted of a refrigerated pre-mix feed preparation drum, a refrigerated feed drum, stainless steel 100 mesh filter on the feed pump intake, a feed pump and rotameter. Both drums were 80-L 316 stainless steel (SS) drums equipped with tight fitting lids to minimize volatilization losses. The pre-mix drum was used to dissolve nutrients and test compounds. The premix drum was equipped with a 1/30 hp variable speed agitator and four 2.54-cm baffles.

Both reactors were fed from a common feed drum located in a refrigerator which was maintained at approximately 1.5°C. Intakes to the suction lines

TABLE 81. MATERIAL BALANCE FOR PRIORITY POLLUTANTS IN BATCH REACTORS*

Compound	Influent (mg/L)	Effluent (mg/L)	Percent overall removal	Percent removed by		
				Stripping	Sorption	Biodegradation
Acrylonitrile	110	1.0	99.1	25.0	0.0	75.0
2,4-Dichlorophenol	130	48.0	63.0	0.0	0.0	100.0
Phenol	100	1.0	99.0	0.0	0.0	100.0

*See Refs. 4, 6, and 19.

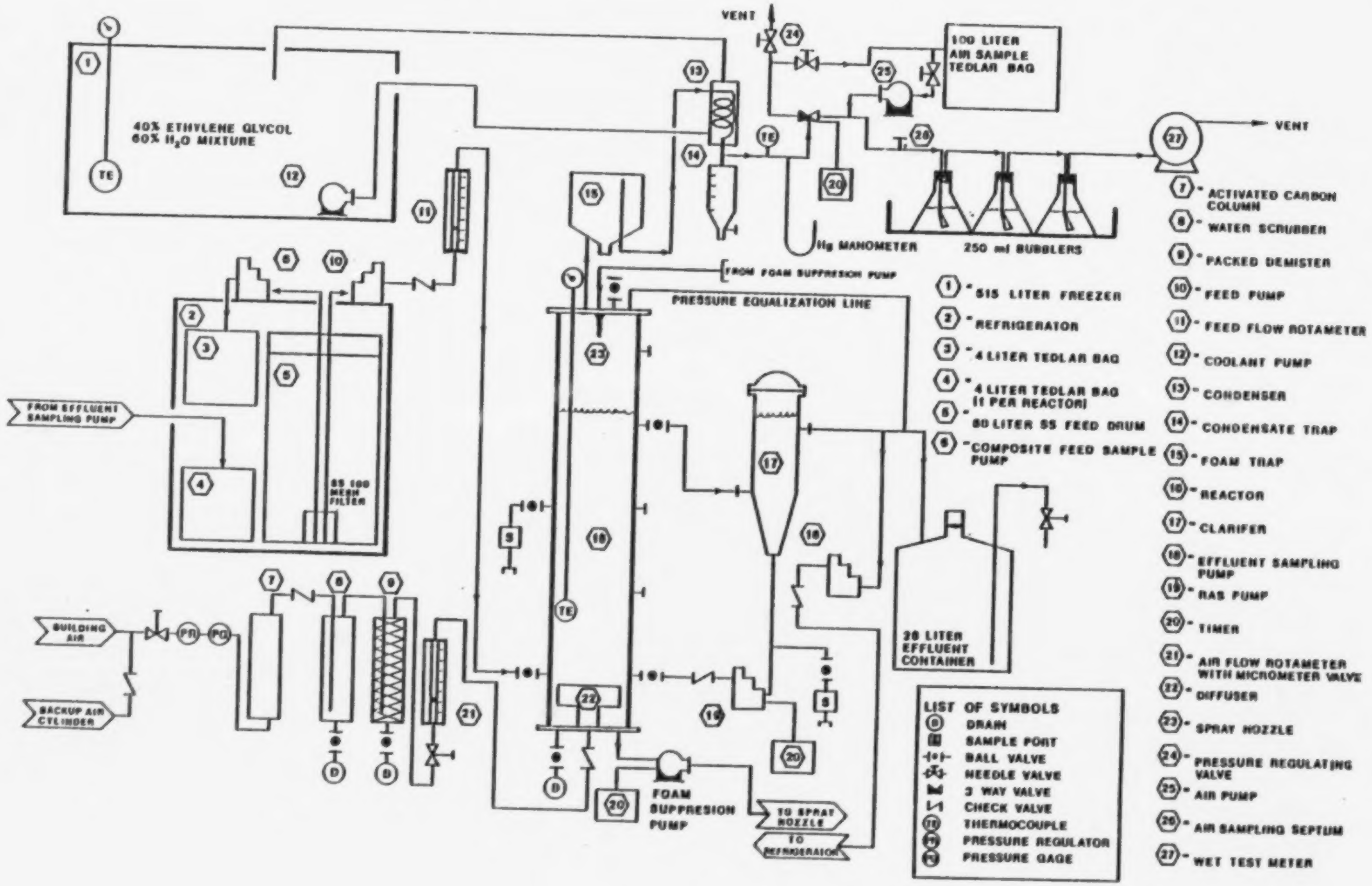
VENT
100 LITER AIR SAMPLE TEDLAR BAG
VENT
40% ETHYLENE GLYCOL 60% H₂O MIXTURE
TE
Hg MANOMETER
250 ml BUBBLERS
FROM FOAM SUPPRESION PUMP
PRESSURE EQUALIZATION LINE
FROM EFFLUENT SAMPLING PUMP
SS 100 MESH FILTER
BUILDING AIR
BACKUP AIR CYLINDER
PR
PG
S
D
28 LITER EFFLUENT CONTAINER
FOAM SUPPRESION PUMP
TO SPRAY NOZZLE
TO REFRIGERATION
1 - 515 LITER FREEZER
2 - REFRIGERATOR
3 - 4 LITER TEDLAR BAG
4 - 4 LITER TEDLAR BAG (1 PER REACTOR)
5 - 80 LITER SS FEED DRUM
6 - COMPOSITE FEED SAMPLE PUMP
7 - ACTIVATED CARBON COLUMN
8 - WATER SCRUBBER
9 - PACKED DEMISTER
10 - FEED PUMP
11 - FEED FLOW ROTAMETER
12 - COOLANT PUMP
13 - CONDENSER
14 - CONDENSATE TRAP
15 - FOAM TRAP
16 - REACTOR
17 - CLARIFER
18 - EFFLUENT SAMPLING PUMP
19 - RAS PUMP
20 - TIMER
21 - AIR FLOW ROTAMETER WITH MICROMETER VALVE
22 - DIFFUSER
23 - SPRAY NOZZLE
24 - PRESSURE REGULATING VALVE
25 - AIR PUMP
26 - AIR SAMPLING SEPTUM
27 - WET TEST METER
LIST OF SYMBOLS
D DRAIN
S SAMPLE PORT
BALL VALVE
NEEDLE VALVE
3 WAY VALVE
CHECK VALVE
TE THERMOCOUPLE
PR PRESSURE REGULATOR
PG PRESSURE GAGE

FIG. 51. LAB-SCALE ACTIVATED SLUDGE UNITS

for reactor feed pumps and the composite feed sample pump were enclosed in a SS 100 mesh filter. Feed was pumped from the feed drum to the biological reactors by a Gorman Rupp Model No. 14280 bellows pump. A rotameter in the feed line between the pump and the reactor was used to monitor the feed flow rates.

Biological Reactor and Clarifier --

The biological reactor was constructed of a 91.44 cm (36-in.) long by 15.24 cm (6-in.) ID Pyrex glass column. Total volume of each reactor was 16.68 L; however, reactors were operated at a liquid volume of 11 L to allow head space for foam separation at the top of the column. Seven Ace glass threaded connectors were provided on the Pyrex column at the locations shown in Figure 51.

Top and bottom reactor flanges were fabricated of 316 stainless steel (SS) and were sealed to the Pyrex columns with collar and bolt assemblies which compressed O rings between the glass and metal surfaces. The top flange was provided with an exhaust gas port, a pressure equalization port, a spray recycle port, a thermocouple port, and a ball valve. The bottom flange was provided with a drain port and ports for the diffused air and foam suppression intake line connections.

The diffuser was constructed of a 316 SS pipe cap with a milled surface. The top surface of the diffuser was mounted 11.4 cm above the base of the column. Four equally spaced 0.08-cm (1/32-in.) diameter orifices were drilled at a 2.54-cm radius from the center of the diffuser.

An external clarifier was fabricated from 10.2-cm ID Pyrex pipe. The clarifier had a side water depth of 33 cm and a liquid volume of 1.5 L. The clarifier was equipped with a lid which was sealed with an O-ring and U-clamp.

Sludge was recycled from the clarifier to the reactor by a variable stroke pump which was controlled by a timer. The timer was adjustable to allow return activated sludge (RAS) pumping to vary from 0 to 100% of the time the system was in operation.

Effluent from the clarifier flowed by gravity to an air-tight 28-L polyethylene carboy. A pressure equalization line was provided between the effluent line and the reactor in order to eliminate pressure differences in the reactor, clarifier, and effluent container due to pressure changes in the air sampling system.

Feed, Effluent, and Mixed Liquor Sampling System --

The suction end of the intake line for the composite feed pump was enclosed in the same 100 mesh SS cartridge filter with the reactor feed lines. A continuous sample was pumped to a 4-L Tedlar bag located in the refrigerator.

Effluent samples were continuously pumped from the clarifier overflow line to a 4-L Tedlar bag located in the refrigerator. All samples for test compound and ^{14}C analyses of effluent samples were obtained from this composite sample. Samples for effluent total suspended solids (TSS) analysis were obtained from the 28-L effluent container.

Grab samples of mixed liquor were obtained from the sampling port on the reactor.

Exhaust Gas Sampling System --

Exhaust gas from the reactor passed through a 250-mL polyethylene foam trap and then to a condenser. The condenser was operated in a counter-current mode by circulating a 40% ethylene glycol/60% water mixture at a temperature which varied from 6 to 9°C. Condensate was collected in a graduated 100-mL condensate trap. Temperature and pressure of the exhaust gas were measured at the exit of the condenser. The exhaust gas was then directed to a timer actuated three-way valve. The timer had a total cycle time of 30 min with 15 sec increments. The timer/valve combination was adjustable to direct 0 to 100% of the exhaust gas to one of two branches of the exhaust gas sampling system.

One branch of the air sampling system consisted of a line equipped with an air sampling septum followed by three 250-mL ASTM 40/60 fritted glass bubblers. Each bubbler was filled with 150 mL of 0.5 N NaOH at the beginning of each sampling run. A wet test meter was connected to the exhaust line from the last bubbler. This sampling train was used to collect composite samples of $^{14}CO_2$, phenol, aniline, and pentachlorophenol.

The second branch of the air sampling train consisted of a 100-L Tedlar bag. This system was used for collecting samples at near atmospheric pressure. Samples collected in the bag were then pumped back to the branch of the gas sampling train containing the sampling septum and bubblers. This procedure was used for collecting gas samples for the analysis of toluene.

Experimental Procedures

Overview --

Lab-scale activated sludge studies were conducted for four compounds which were individually spiked into a foul condensate wastewater. Selected physical properties are included in Table 82.

TABLE 82. COMPOUNDS STUDIED IN LAS SYSTEMS AND THEIR SELECTED PHYSICAL/CHEMICAL PROPERTIES

Compound	Henry's law constant (Torr·L/g-mole)	Octanol/water partition coefficient
Phenol	0.25	30.9
Toluene	2,769	537
Aniline	0.76	7.9
Pentachlorophenol	2.10	132,000
Pentachlorophenate at pH=7		7700

Sludge for seeding the LAS reactors at the beginning of an experiment for each compound was obtained from acclimation and seed reactors (ASR) which have been described previously in Section 5 of this report. ASRs were operated in a fill-and-draw mode. Each LAS reactor was seeded with sludge from an ASR reactor which had been operated at an equivalent mean cell residence time. Total acclimation time for the biomass in the ASRs for the phenol, toluene, aniline, and pentachlorophenol experiments was 149, 196, 25, and 27 days, respectively.

LAS reactors for the phenol, toluene, aniline, and PCP experiment were operated for 33, 8, 8, and 6 days, respectively, before a ^{14}C labeled compound was introduced into the feed and the entire sampling and analytical program was implemented. Minimal acclimation time was allowed in the LAS reactors since stable biological populations had previously been developed in the ASRs. The schedule for the experimental program for each of the test compounds is shown in Table 83. Unless otherwise noted, the discussion of the results of the LAS study is limited to data collected during the time ^{14}C labeled substrates were being fed to the systems.

TABLE 83. EXPERIMENTAL PROGRAM SCHEDULE FOR LAS STUDIES

Test compound	Date LAS reactor seeded	Date ^{14}C labeled compound introduced	Date LAS reactors shut down
Phenol	04-28-83	06-01-83	06-08-83
Toluene[a]	06-14-83	06-22-83	07-22-83
Aniline	07-23-83	07-29-83	08-12-83
Pentachlorophenol	08-18-83	08-22-83	09-02-83

[a] Limited analytical data were collected during the period 07-02-83 to 07-11-83.

The full sampling and analytical protocol was not followed during the period 07-02-83 to 07-11-83 due to vacation time taken by the operations staff during this period. Reactors were fed foul condensate spiked with 200 mg/L toluene were monitored to maintain the target mean cell residence times. No ^{14}C labeled toluene was fed during this period.

Design parameters for the LAS systems are as follows:

Feed flow	11 liters/day
Hydraulic residence time	24 hours
Air flow	2 liters/min
Mixed liquor temperature	20°C
Dissolved oxygen	>2 mg/L
pH	6.5 to 7.5
Mean cell residence time	5 days (Reactor No. 1) 10 days (Reactor No. 2)

Operating Procedures --

Feed flow rates were determined by a rotameter on the feed line to each reactor. Feed volumes recorded in the data log for each feed period were determined by weighing the effluent collected in the effluent drum and the effluent sampling bag. Air flow rates were controlled by a rotameter on the air supply line. All air flow rates are reported at ambient temperature and pressure. Rotameters were calibrated with a wet test meter located on the vent discharge line.

Both the return activated sludge (RAS) pump and the foam suppression pump were operated by timer systems. The return activated sludge pumps were set to operate for 1 min out of every 5 min. RAS flow rate during operation was approximately 50 mL/min yielding an effective average flow of approximately 14 L per day. This mode of operation was chosen over a continuous flow at a lower rate. An intermittent flow at a higher rate was found to be more effective in removing solids from the clarifier due to a higher velocity gradient at the RAS pump intake.

The foam suppression spray system was operated during the phenol study and was then discontinued in favor of using an antifoaming agent. The foam suppression pump was set to operate 1 min out of every 4 min. The flow rate during operation was approximately 1.2 L/min for reactor 1 and 2.4 L/min for reactor 2.

Mean cell residence time (MCRT) was controlled by removing mixed liquor on a daily basis directly from the reactors. Mixed liquor was removed from the reactors according to the following relationship:

$$V_w = \frac{\dfrac{(V \cdot MLSS) + (V_f \cdot TSS)}{MCRT} - (V_f \cdot TSS)}{MLSS} \qquad \text{Eqn. 60}$$

where:

V_w = volume of mixed liquor to be removed from reactor (liters),
V = volume of reactor (lit),
MLSS = mixed liquor suspended solids (mg/L),
V_f = volume of feed during sampling period (liters),
TSS = effluent total suspended solids (mg/L), and
MCRT = mean cell residence time (days).

This equation is based on the assumption that the active mass of biological solids includes the total mass of cells in the reactor, clarifier, and effluent receiver. The cells in the effluent receiver resided in the reactor system for one-half of the time, on the average.

Mixed liquor removed from the reactors was settled for 30 min in a separatory funnel. Solids were drawn off of the bottom and wasted, and the supernatant was returned to the reactors through a ball valve located on the reactor lids. The volume of sludge wasted was recorded in the data log.

Feed Preparation --

Feed to the LAS units consisted of foul condensate from a kraft pulp and paper mill spiked with the test compound, inorganic nutrients, and a phosphate buffer system. Sources, grades, and target concentrations of the test compounds in the feed are shown in Table 84, and the characteristics of the ^{14}C labeled compounds used in this study are shown in Table 85. Criteria which were used in selecting the concentrations of the test compounds included:

- Solubility
- Toxicity
- Available data on tendency to strip, sorb, or biodegrade
- Analytical detection limits

TABLE 84. TEST COMPOUND CHARACTERISTICS

Compound	Vendor	Grade	Target concentration[a] in feed (mg/L)
Phenol	Fisher	ACS	1000
Toluene	J.T. Baker	Distilled in glass	200
Aniline	J.T. Baker	Reagent	100
Pentachlorophenol	Fluka AG	>99% pure	10

[a] Only 1 test compound was added to the feed during the study for that compound.

TABLE 85. ^{14}C LABELED TEST COMPOUND CHARACTERISTICS

Compound	Vendor	Specific[a] activity (μ curies/μ mole)	Purity (%)
Phenol	Amersham, Inc.	71.1	99.0
Toluene	Pathfinder Laboratories	5.11	>98.0
Aniline	Amersham, Inc.	102	98.4
Pentachlorophenol		4.43	

[a] 1 μ curie is equivalent to 2.2 X 10^6 disintegrations per minute (DPM). All compounds were uniformly labeled with ^{14}C.

Feed concentrations were chosen at relatively high levels in order to attempt to provide concentrations in the effluent vent gas and sludge which were above analytical detection limits. Detailed information on analytical detection limits for each analysis is given in Appendix B.

Inorganic compounds which were added to the feed are shown in Table 86. The foul condensate was a product of the contact condensation of a distillate stream. Concentrations of trace metals that were present in the foul condensate have been discussed in Section 5 of this report.

TABLE 86. INORGANIC NUTRIENT CONCENTRATION IN FEED

Compound	Concentration in feed (mg/L)
$CaCl_2 \cdot 2\ H_2O$	132
NaCl	100
$MgSO_4 \cdot 7\ H_2O$	175
NH_4NO_3	Variable
K_2HPO_4	Variable
KH_2PO_4	Variable

A buffer solution consisting of a 2:1 molar ratio of K_2HPO_4: KH_2PO_4 was used to maintain the system pH between 6.5 and 7.5. The quantity of buffer solution added to the feed varied depending upon the characteristics of the feed and daily pH readings in the reactors. Typical concentrations in the feed were approximately 1000 mg/L K_2HPO_4 and 400 mg/L KH_2HPO_4.

Ammonium nitrate was added to the feed to maintain a weight ratio of 5 parts of TOC to 1 part of NH_3N.

The foul condensate wastewater had a tendency to foam excessively when aerated. In order to minimize the loss of the test compound by foam fractionation and to prevent foam carryover into the air sampling system, an antifoaming agent was used during LAS studies with toluene, aniline, and pentachlorophenol. Dow-Corning 1520 silicon antifoam was added to the feedstock at a concentration of 44 mg/L during studies with these three compounds.

Feed to the system was prepared in 75 liter batches which was sufficient to feed two reactors for approximately 3 days. Foul condensate, the test organic compound, nutrients, buffers, and antifoam were mixed for 24 hr in an 80-L 316 SS drum for 24 hr prior to being transferred to the feed drum. The pre-mix drum was maintained in a cooler at 4°C. Residual liquid in the feed drum was discarded every third day, and fresh feed was pumped from the premix drum to the feed drum.

Analytical Methods --

Major analytical equipment used in the LAS study is shown in Table 87.

Detailed methods for the analysis of phenol, toluene, aniline, and pentachlorophenol are outlined in Appendix B. Specific compound analyses in most cases was performed using gas or liquid chromatographic techniques that utilized either direct aqueous or direct gas phase sample injection. The methods were chosen or developed to provide valid analytical data for each compound and matrix selected. Concentrations of liquid phase samples were reported in units of mg/L without correction for recovery. Gas phase analyses were reported in units of ppm in air (v/v) of the compound. Bubbler vent gas samples collected in bubblers were reported as total mg of compound recovered in the bubbler train. Sorption of the test compound to biomass was determined by extracting the biomass with basic methanol and reporting the results as ug of compound per gram of dry weight of biomass without correction for recovery.

Detailed methods for the sampling and analysis of radiolabeled compounds are presented in Appendix B. ^{14}C determinations were made by adding an aliquot of a processed sample to an appropriate solvent (Omnifluor or Redisolve depending on sample) and counting the sample on a Tracor Analytic Beta Trac 6895.

Methods for analysis of suspended solids, volatile suspended solids, BOD_5, TOC, and COD were chosen from "Standard Methods for the Examination of Water and Wastewater," 15th edition. All reported values of BOD_5, COD, and TOC are on a soluble basis, i.e., samples filtered through a 0.45 µ filter.

Sampling Protocols --

Sampling locations and analytical parameters measured at each location are shown in Table 88. Sampling methodologies for ^{14}C analyses are presented in Appendix B. Sampling procedures for the analysis of specific compounds are discussed below.

Sampling procedures for phenol and aniline were identical as described:

- Feed-sample collected continuously in 4-L Tedlar bag. Aliquots were transferred into a 20-mL scintillation vial, filtered through 0.45 µ filter paper and analyzed by LC.
- Mixed Liquor -- Grab samples were obtained in a 50-mL conical centrifuge tube after flushing of the sample port line and immediately centrifuged at 1800 rpm for 5 min. A 25-mL aliquot of the supernatant was removed and filtered through 5 µm filter paper prior to analysis by LC.

TABLE 87. LAS STUDY ANALYTICAL EQUIPMENT

Item	Manufacturer	Model
pH meter	Corning	135
Electronic analytical balance	Metler	AC100
Electronic analytical balance	Metler	PC2200
Refrigerated centrifuge	IEC	Centra 7R
Dissolved oxygen meter	YSI	54A
Drying oven	Fisher	100 series 126G
Vacuum pump	GCA Corp. (Precision)	DD50
Wet test meter	GCA Corp. (Precision)	Large 3L face
TOC analyzer	Beckman	915 (IR-865)
Freeze-dryer	Labcono	18
Gas chromatograph	Hewlett-Packard	5880
Portable gas chromatograph (CO_2 analysis)	Analytical Inst. Development	
Scintillation counter	Tracor Analytic	Beta Trac 6895
Differential respirometer	Gilson Medical Electronics	ICR-14

TABLE 88. SAMPLES AND ANALYTICAL PARAMETERS LAB-SCALE ACTIVATED SLUDGE UNIT

Sample Type	Sample location	Analytical parameters
Feed	Composite feed sample bag	Soluble test compound concentration Soluble BOD_5 Soluble TOC NH_3 ^{14}C
Effluent	Composite effluent sample bag	Soluble test compound concentration Soluble BOD_5 Soluble TOC NH_3 Soluble ^{14}C
	28 liter effluent container	Effluent total suspended solids (TSS)
Mixed liquor	Grab sample from sample port on reactor	Soluble test compound concentration Test compound sorbed to activated sludge Soluble ^{14}C ^{14}C sorbed to/incorporated in activated sludge Mixed liquor total suspended solids (MLSS) Mixed liquor volatile suspended solids (MLVSS) pH Dissolved oxygen
Exhaust gas	Condensate trap	Test compound concentration ^{14}C
	Bubblers	Test compound concentration ^{14}C
	Air sampling system	Test compound concentration (toluene) CO_2 concentration

Samples for the determination of the sorption of the test compound to biological solids were further processed by decanting the supernatant and centrifuging the sample for 10 min at 1800 rpm. Any additional supernatant was decanted and a measured volume of basic methanol extracting solution was added to the centrifuge tube. The biomass was stirred into the extracting solution with a Teflon coated spatula and then allowed to remain in contact with the extracting solution for 2 to 3 hours. The mixture was then centrifuged at 1800 rpm for 20 min and the supernatant extract solution was removed into a clean 20-mL scintillation vial. An aliquot of the extract was acidified with a measured volume of 0.1 N HCl solution sufficient to neutralize and acidify the basic methanol extract. The neutralized extract was then filtered through a 0.45 µm filter paper to remove solids and the filtrate.

Effluent -- Samples were collected by a continuous sampling pump into a 4-L Tedlar bag. Aliquots were removed from the bag into a scintillation vial and filtered through 5 µm filter paper and analyzed by direct injection into the LC.

Vent Gas -- Vent gas from the activated sludge system was passed through a three unit bubbler train containing 150 mL of 0.5 N NaOH per unit. Aliquots from each bubbler were acidified with an equal volume of 0.55 N HCl and analyzed by LC. Composite condensate samples were collected from the condensate trap and analyzed as is by LC.

Sampling procedures for the toluene study are as follows:

Feed -- Composite sample collected in 4 L Tedlar bag. An aliquot of the sample from the bag was analyzed by direct aqueous injection on the gas chromatograph without further treatment.

Mixed Liquor -- A grab sample was obtained through the column sample port after flushing of the sample line. The liquid was collected in a scintillation vial with the outlet of the sample line below the surface of the liquid and then the vial was capped with a septum cap. The sample was immediately analyzed by direct aqueous injection into the gas chromatograph without further treatment.

Effluent -- Effluent was composited and refrigerated in 4-L Tedlar bags by a continuous sampling pump. An aliquot of the bag sample was analyzed by direct aqueous injection on the gas chromatograph without further treatment.

Vent Gas -- Grab samples were obtained from a septum system contained in the vent line using a 2-cc gas syringe with a valved tip. The clean syringe was inserted through the septum and the valve opened. The plunger was withdrawn and depressed several times to rinse the syringe with sample and then the plunger was withdrawn to about 1.6 mL and held for 10 sec. The plunger was then depressed to the required volume of 1 cc and held for an additional 10 sec. The valve was then closed and the syringe withdrawn from the system. The sample was then injected into the GC as quickly as possible after sampling to prevent losses.

Since the vent gas stream was passed through a three-unit caustic bubbler train to trap radioactive CO_2, toluene contribution to the radioactivity needed to be determined. Aliquots from each of the bubblers were combined into a single sample and then neutralized with an equal volume of 0.55 N HCl. The combined neutralized sample was then analyzed by direct aqueous injection on the gas chromatograph.

Composite condensate samples were collected from the condensate trap in a scintillation vial and immediately analyzed in the GC by direct aqueous injection.

Sampling procedures for the pentachlorophenol study are as follows:

Feed -- A grab sample of the feed was taken from the feed drum in a 50-mL tared conical tip centrifuge tube, capped and centrifuged at 1800 rpm for 15 min. The supernatant was filtered through 0.45 μm filter paper and analyzed by LC.

Mixed Liquor -- A grab-sample was obtained through the column sample port after flushing the sample line. Mixed liquor samples were taken as 40-mL samples in 50-mL conical tip centrifuge tubes. Samples were centrifuged at 1800 rpm for 15 min. The supernatant was removed and filtered through 0.45 μm filter paper prior to analysis.

Pentachlorophenol adsorbed to biological solids was determined by recording the volume of biological solids remaining in the centrifuge tubes and extracting the biomass with basic methanol for 12 to 16 hours. The sample was then centrifuged at 1800 rpm for 15 min. The supernatant was acidified, filtered through 0.45 μm filter paper and analyzed by LC.

Effluent -- Composite samples were collected in 4-L Tedlar bags. Aliquots of the effluent were taken in 50-μL centrifuge tubes and handled by the same procedures used to handle the feed sample.

Vent Gas -- Samples were handled by the same procedures previously described for phenol and aniline.

A "daily sampling period" was defined as the elapsed time between the beginning of composite feed and effluent sample collection and the time that the composite samples were removed from the system. Sampling periods were approximately 24 hr long during the work week but varied from 17 to 32 hr on weekends.

Samples of feed effluent and mixed liquor were collected at approximately 8:00 a.m. each weekday. Sampling pumps were turned off and the composite sample bags disconnected from the sampling lines. Samples were split into appropriate sample containers depending upon which analyses were to be performed on a given day. Sample bags were rinsed with deionized water and reconnected to the sample lines.

Mixed liquor grab samples for suspended solids, DO, and pH analyses were taken immediately after composite feed and effluent samples were collected. Mixed liquor samples were collected in 500-mL glass beakers. The sample line on the reactor was flushed with a volume of mixed liquor greater than three times the sample line volume before the sample was collected. The mixed liquor sample was withdrawn slowly and with the tip of the sample line submerged in order to minimize turbulence and volatilization losses. A 25-mL sample of mixed liquor was obtained at the same time for analysis of the number of organisms per mL capable of degrading the test compound. This sample was collected in a 25-mL glass vial with a Teflon lined screw cap and was refrigerated with ice and transported immediately to the University of Tennessee for analysis.

Effluent collection drums were emptied at the same time that composite feed and effluent samples were being taken. The pressurized effluent collection containers were emptied by opening a valve which was between a Teflon tube extending to the bottom of the container and a 2-ft length of rubber tubing which was inserted into a 30-L polyethylene transfer vessel. The liquid collected in the transfer vessel was weighed and the time at which the effluent was collected was recorded. The transfer vessel was agitated and a 100-mL sample was poured into a 200-mL glass beaker. This sample was analyzed for effluent total suspended solids.

Design of the vent gas sampling system allowed for a composite gas sample to be collected by directing the vent gas stream to the bubblers or the vent by means of a timer and three-way valve. The timer used had a 30-sec cycle time with 15 sec increments. The fraction of the total air flow to be sampled was to be set to prevent breakthrough of CO_2 into the third bubbler. This design proved unsatisfactory due to difficulties in balancing the pressure drop between the bubblers and the vent. When the vent gas was directed to the bubblers, a significant amount of time elapsed between the time the bubblers were switched on line and the time that sufficient pressure built up in the system to overcome the pressure drop in the bubblers (approximately 165 mm of Hg at 2 L/min air flow). Three alternatives were available to rectify the problem. One alternative would have been to provide a parallel set of bubblers on the vent discharge. The second alternative was to provide a timer with a longer cycle time so that the ratio of non-sampling time to sampling time was small when the bubblers were switched on. The third alternative (and the one chosen) was to set the three-way valve to direct all vent gas through the bubblers and sample continuously for a specified period each day. "Air sampling periods" for all compounds except toluene were either 2.5 or 3 hr periods. Vent gas emissions in a "daily sampling period" have been calculated by the following equation:

$$M_T = M_B \times \frac{DSP}{ASP} + M_c \qquad \text{Eqn. 61}$$

where:

M_T = total mass of air emissions in "daily sampling period" (mg),
M_B = total mass of emissions collected in bubblers in "air sampling period" (mg),
M_C = total mass of emissions collected in condenser during "daily sampling period" (mg),
ASP = air sampling period (hours), and
DSP = daily sampling period (hours).

The above calculation reflects that the condenser was continuously on line in the air sampling stream.

Toluene was the most volatile of the compounds studied and was expected to be significantly removed from the LAS system by the stripping mechanism. Sampling and analytical protocol development prior to the initiation of the sampling campaign for the LAS toluene study revealed that the mass emission rate of toluene from the reactors was extremely sensitive to changes in the operating system pressure. Figure 52 illustrates the variation in vent gas toluene concentration that occurred following the release of pressure on the reactor system. Both reactors had been placed under approximately 165 mm Hg pressure for 3 hr by diverting the vent gas through the bubbler train. At time zero pressure was released from both systems by diverting the vent gas to a vent at atmospheric pressure. Later in the toluene run conditions were duplicated in order to confirm the pressure effect. However, the result was not nearly as apparent. Apparently, the pressure transient affected the biotransformation kinetics early in the run allowing more material to be available for trapping. Population or enzyme changes presumably mitigate this effect as the biomass turns over. In any case, the potential for large variations in mechanism kinetics as a result of system transients is clearly demonstrated.

Due to the large variation in stripping rate caused by variable pressure on the reactor system, an operational and sampling protocol was developed to collect vent gas samples for the toluene study at atmospheric pressure. Vent gas samples were collected for 40 min in a 100-L Tedlar bag. The contents of the bag were then pumped to the bubbler train to collect $^{14}CO_2$. Vent gas samples for the analysis of toluene were withdrawn through a septum located in front of the bubbler train. A wet test meter on the discharge side of the bubblers was used to measure the volume of the air sample.

RESULTS AND DISCUSSION

Overview

Appendix G contains daily operating parameters and analytical data for the LAS study. A summary of the system operating parameters is shown in

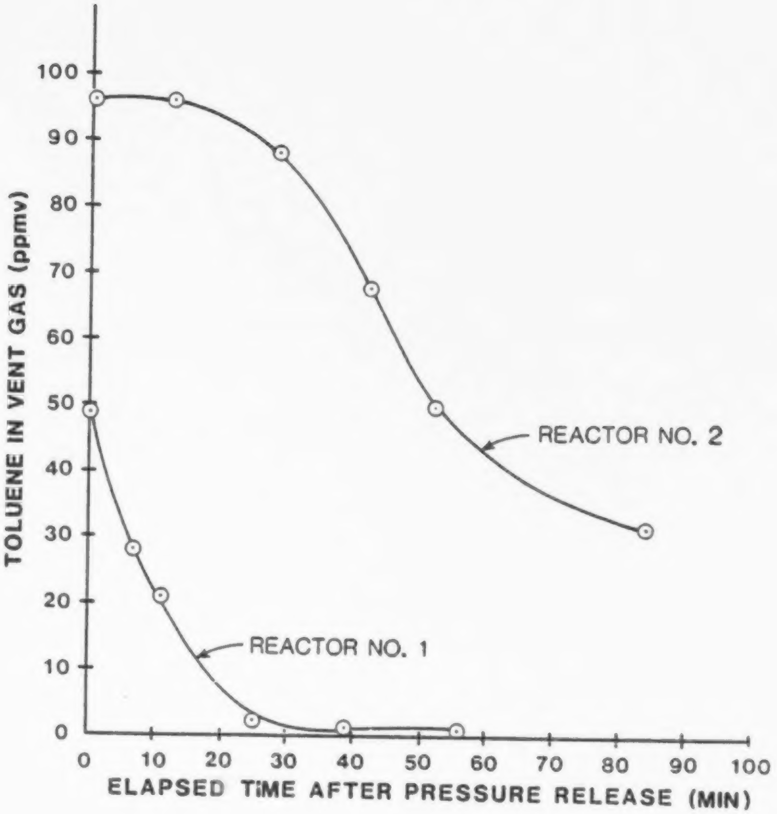

FIG. 52. VARIATION IN VENT GAS TOLUENE CONCENTRATION AFTER RELEASE OF PRESSURE ON REACTOR

Table 89, and a summary of substrate removal data is shown in Table 90. Examination of the data in Table 90 indicates very high overall removal efficiencies for phenol, toluene, and aniline while pentachlorophenol was found to be very resistant to removal from the process wastewater.

Material balances based on specific compound analysis are presented in Table 91, and material balances based on ^{14}C analysis are presented in Table 92. Removal of the test compounds by bio-oxidation was calculated by subtracting the mass of material recovered in the effluent, vent gas, and waste solids from the mass of compound in the feed. Sorption of toluene to the mixed liquor suspended solids was not measured; therefore, the material balance cannot be closed for this compound.

Waste solids were assumed to have the same chemical composition as mixed liquor suspended solids (MLSS), i.e., the compound loading and ^{14}C analyses of effluent suspended solids and waste activated sludge solids were assumed to be the same as measured for the MLSS.

Data in Table 92 do not differentiate among the possible fates of ^{14}C; the ^{14}C may be present as the original compound, a metabolite, $^{14}CO_2$, or as cell mass. However, data in Table 91 show that for phenol, toluene, and aniline, very small percentages of the parent compound were recovered in either the waste solids or the effluent, indicating that the ^{14}C was not recovered in the form of the parent compound in these sampling locations. Therefore, it appears that a large percentage of the ^{14}C recovered from biological solids was converted into cellular mass. For these three test compounds, soluble ^{14}C in the sludge and effluent is presumed to be predominantly in the form of a metabolites of the parent compound. Greater than 99% of the ^{14}C collected in the bubblers was precipitated as $BaCO_3$ for selected samples, indicating that virtually all of the ^{14}C collected in the bubblers was from $^{14}CO_2$ rather than a volatile metabolite. Both the test compound and ^{14}C material balances for pentachlorophenol show that approximately 6 to 8% of the feed compound was sorbed to the waste solids and greater than 73% of the feed pentachlorophenol was found in the effluent.

Coefficients of variability were calculated for key operating parameters for each reactor for studies on all four compounds. The results of the coefficient of variability study for the parameter MLSS and effluent TOC indicate that all reactors were operating at a relatively steady-state condition with respect to these two parameters. Coefficients of variability for the MLSS concentration ranged from a low of 6.0% for aniline reactor number 1 to a high of 26.8% for PCP reactor number 1. The average coefficient of variability of MLSS concentration for all reactors during studies with the four compounds was 12.6%.

Although in the cases of non-^{14}C-labeled substrate the systems were each run at significant multiples of the MCRT and can be assumed to be at steady-state, the addition of ^{14}C substrate occurred over 7, 10 and 11, 15, and 12 days for phenol, toluene, aniline, and PCP, respectively. The time that radiolabeled compounds were fed into reactor 1 ranged between 1.4 and 3.0

TABLE 89. SUMMARY OF LAB-SCALE ACTIVATED SLUDGE UNITS AVERAGE OPERATING PARAMETERS

Test compound	Reactor number	Mean cell residence time (days)	Feed flow (L/day)	Hydraulic residence time (hr)	MLSS (mg/L)	MLVSS (mg/L)	pH	Mixed liquor temperature (°C)	Mixed liquor DO (mg/L)	Air flow (L/min)
Phenol	1	4.8	9.4	28.0	2,220	1,960	7.00	22.2	5.2	2.50
	2	8.8	10.2	26.0	3,650	3,350	6.95	22.6	4.9	2.43
Toluene	1	4.6	10.5	22.8	1,280	1,170	6.96	20.6	3.5	2.03
	2	9.1	11.2	23.6	2,370	2,110	6.98	21.1	2.1	2.04
Aniline	1	5.0	11.3	23.3	1,690	1,530	6.82	21.1	5.4	2.08
	2	10.0	11.1	23.8	2,550	2,320	6.79	21.1	4.5	2.04
Pentachlorophenol	1	4.9	10.4	25,4	1,370	1,280	6.80	22.0	6.5	2.01
	2	9.3	11.0	24.0	2,290	2,070	6.81	21.7	5.6	2.02

TABLE 90. SUMMARY OF LAB-SCALE ACTIVATED SLUDGE UNITS AVERAGE SUBSTRATE REMOVAL PERFORMANCE

Test compound	Reactor number	Test compound				Soluble TOC			Soluble BOD$_5$		
		Influent (mg/L)	Mixed liquor (mg/L)	Effluent (mg/L)	Removal efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Removal efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Removal efficiency (%)
Phenol	1	986	<0.50	<0.50	>99.95	1330	240	82.0	2390	75	96.9
	2	986	<0.50	<0.50	>99.95	1330	183	86.3	2390	44	98.2
Toluene	1	195	<0.30	<0.06	>99.97	530	133	74.7	717	28	96.1
	2	195	<0.26	<0.29	>99.85	530	142	73.0	717	31	95.7
Aniline	1	190	<0.10	<0.10	>99.91	460	135	70.7	665	7	98.9
	2	190	<0.10	<0.10	>99.91	460	127	72.4	665	8	98.8
Pentachlorophenol	1	8.6	7.4	7.6	11.6	540	196	63.4	895	123	86.3
	2	8.6	7.3	8.0	7.0	540	165	69.2	895	34	96.2

TABLE 91. TEST COMPOUND MATERIAL BALANCE SUMMARY FOR LAS STUDY

				Percent of Feed					
Test Date	End Date	Test Compound	Reactor No.	Bio-oxidation	Sorbed to[b] Waste Solids	Soluble In Waste Activated Sludge	Soluble Effluent	Vent Gas	Percent of Feed Compound Recovered
06-01-83	06-08-83	Phenol	1	>99.92	0.007	<0.002	<0.052	<0.017	<0.078
			2	>99.93	0.002	<0.002	<0.050	<0.016	<0.070
06-22-83	07-02-83[a]	Toluene[a]	1			<0.001	<0.04	<11.6	
			2			<0.001	<0.16	<11.9	
07-29-83	08-12-83	Aniline	1	>99.88	0.01	<0.003	<0.07	<0.04	<0.12
			2	>99.88	0.01	<0.001	<0.07	<0.04	<0.12
08-22-83	09-02-83	Penta-chloro-phenol	1	0.00	7.45	4.23	89.7	<0.23	102
			2	0.00	6.61	2.51	94.6	<0.22	104

[a] Does not include data for period 07-02-83 through 07-11-83. Units were operated in a maintenance mode and limited analytical data were collected during this period.

[b] Includes effluent TSS and waste activated sludge.

TABLE 92. ^{14}C MATERIAL BALANCE SUMMARY FOR LAS STUDY

Start date	End date	Test compound	Reactor number	Percent of feed					Percent of feed ^{14}C recovered
				Waste[b] solids	Soluble in waste activated sludge	Soluble effluent	Vent gas	Remaining in reactor and clarifier at end of run	
06-01-83	06-08-83	Phenol	1	17.7	0.38	14.0	55.3	10.9	98.4
			2	12.6	0.50	13.2	55.8	15.9	98.0
06-22-83	07-22-83	Toluene	1	25.0	0.17	5.07	67.5	2.78	101.0
			2	16.5	0.73	5.97	70.4	4.13	97.7
07-29-83	08-12-83	Aniline	1	17.7	0.56	14.0	55.6	10.5	98.3
			2	15.9	0.31	12.3	57.3	13.8	99.8
08-22-83	09-02-83	Pentachlorophenol	1	7.8	2.40	73.0	0.50	9.36	93.0
			2	6.07	1.47	78.2	0.81	10.8	97.3

[a] Does not include data for period 07-02-83 through 07-11-83. Units were operated in a maintenance mode and limited analytical data were collected during this period.

[b] Includes effluent TSS and waste activated sludge.

times its MCRT, while similar time that radiolabeled compounds fed into reactor 2 ranged from 0.7 to 1.5 times its MCRT. The solids ^{14}C data shown in specific compound ^{14}C material balance data lead to the conclusion that the midpoint solids loading concentrations are within 90% of the endpoint values (or the average of the values between the midpoint and endpoint) for three-quarters of the cases (reactor 1 - phenol, toluene, and aniline; reactor 2 - toluene, aniline, and PCP). It is probable, therefore, that the estimates of sorption fates using ^{14}C methods are low by 10 to 30% but this level of discrepancy is not likely to be statistically significant and would affect neither the sorption conclusions nor the estimates of other fate mechanisms. Running the ^{14}C intervals of each experiment longer could not be justified and therefore ^{14}C intervals were limited to 1 to 2 weeks duration.

Phenol --

LAS units were started up with a feed phenol concentration of 100 mg/L. Batch studies at the University of Tennessee (Section 9) had indicated increasing rates of phenol mineralization at initial concentrations up to 120 mg/L with decreasing rates at higher concentrations. Phenol in the effluent samples from both LAS reactors was below the detectable limit of 0.5 mg/L at a feed concentration of 100 mg/L. The concentration of phenol in the feed was then stepped up to 1500 mg/L over a period of 11 days as shown in Fig. 53. Effluent phenol concentrations continued to be below detectable limits. However, a sludge bulking problem occurred in reactor number 1 at a feed concentration of 1500 mg/L, and biological solids could not be maintained in the system. The feed concentration was reduced to 1000 mg/L, and units were operated at that feed concentration for the remainder of the LAS study with phenol.

Operating summaries for key parameters for reactors 1 and 2 are shown in Figs. 54 and 55, respectively. Daily material balance summaries for phenol and ^{14}C are shown in Tables 93 and 94.

The performance of reactors operated at mean cell residence times of 4.8 and 8.8 days was almost identical in terms of both phenol removal and the fate of ^{14}C. Phenol was never detected in the effluent, the condenser, or the bubblers for either reactor at an analytical detection limit of 0.50 mg/L, indicating that the parent compound was rapidly biotransformed. Data in Table 92 indicate that approximately 55% of the feed ^{14}C was recovered as $^{14}CO_2$ while approximately 13 to 14% was recovered as a soluble metabolite in the effluent and 13 to 18% was recovered in the waste solids. Average phenol loadings on biological solids were less than 108.5 ug/g and 47.8 ug/g MLSS for reactors 1 and 2, respectively.

Toluene --

Toluene was chosen as a model compound to study the relative rates of stripping and biodegradation. Sorption to solids was not measured for toluene. Since sorption to solids was not measured, no estimate of the percent of the feed toluene bio-oxidized could be made based on the conven-

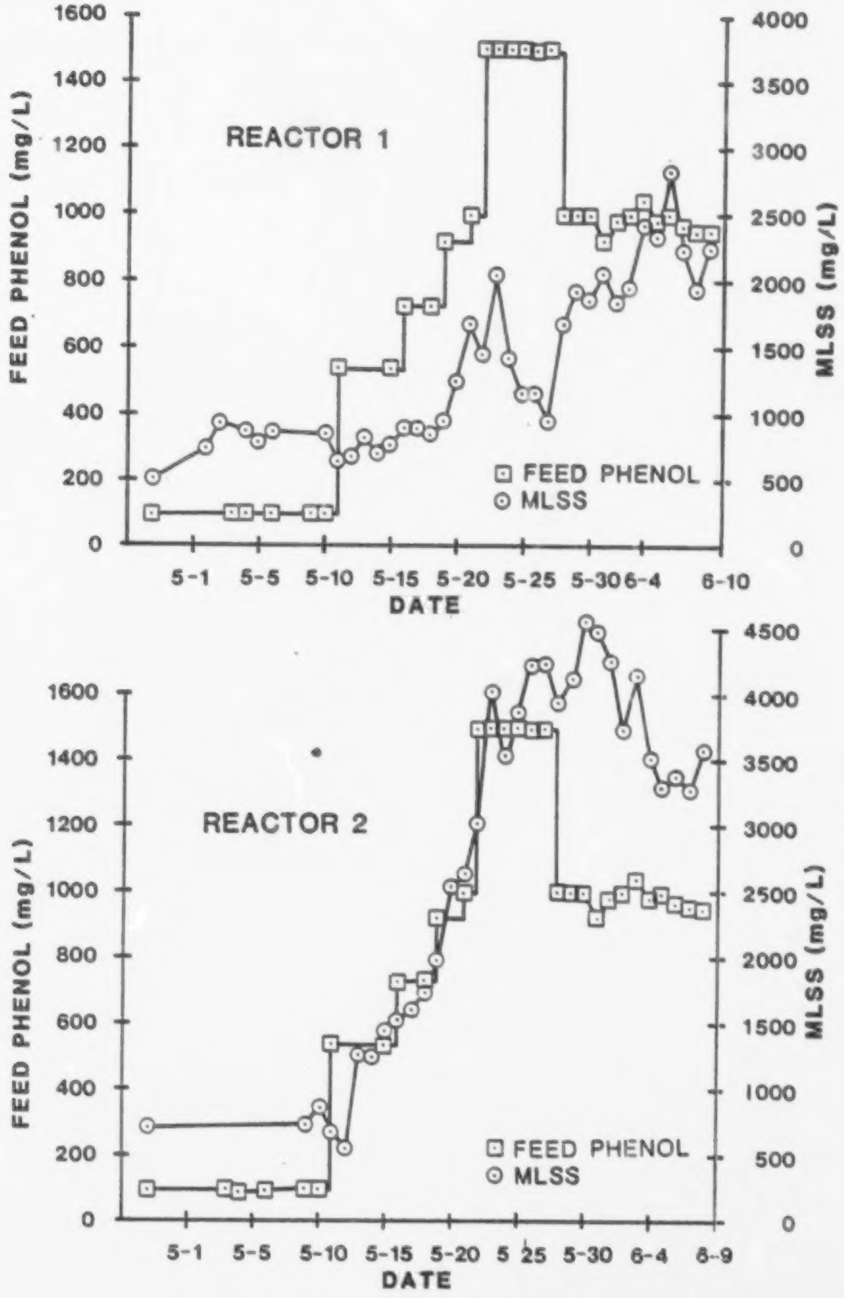

FIG. 53. RELATIONSHIP BETWEEN PHENOL FEED CONCENTRATION AND MLSS

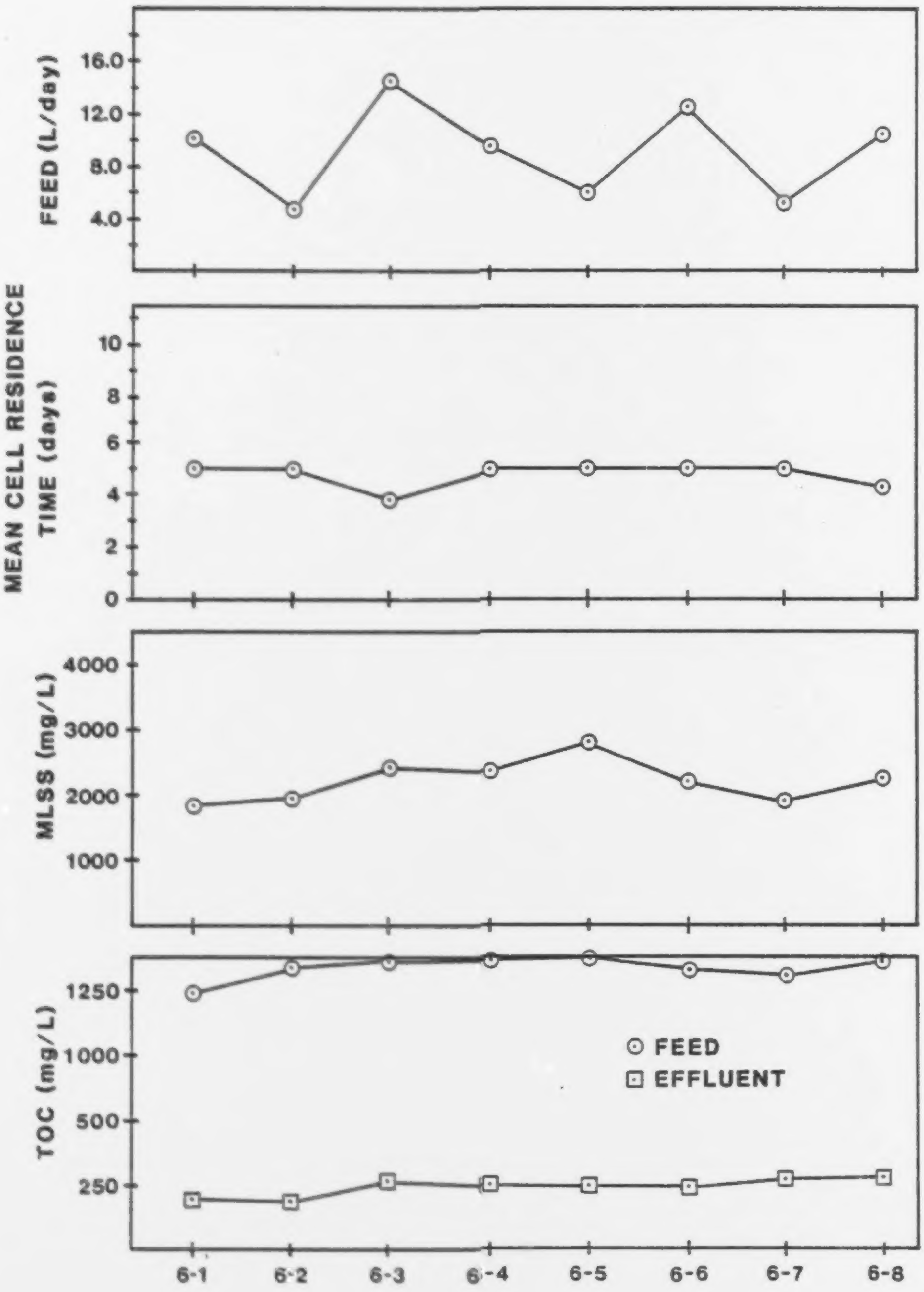

FIG. 54. OPERATING SUMMARY - PHENOL REACTOR NO. 1

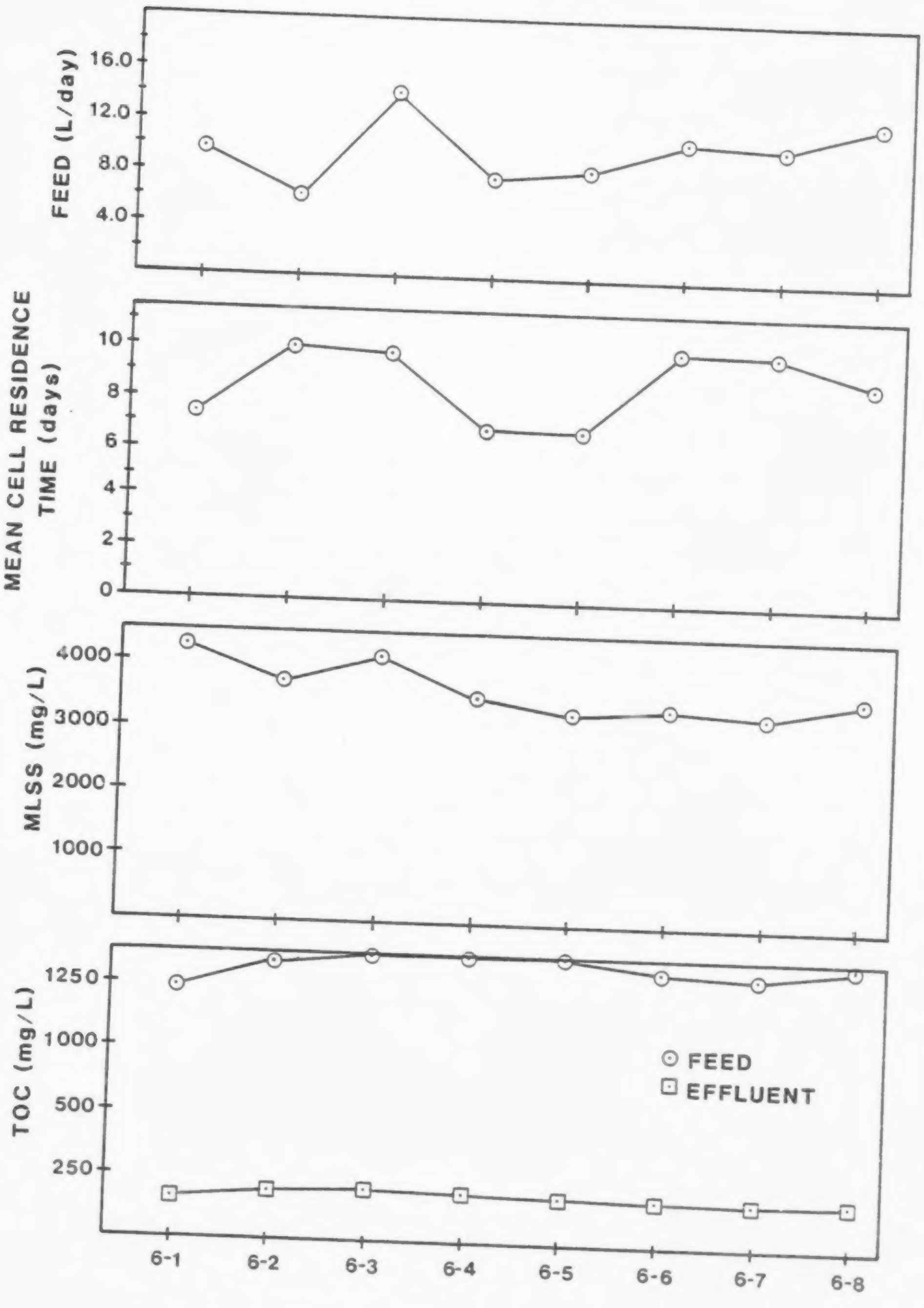

FIG. 55. OPERATING SUMMARY - PHENOL REACTOR NO. 2

TABLE 93. MATERIAL BALANCE SUMMARY FOR LAS UNITS PHENOL CONVENTIONAL ANALYSIS

		Feed				Waste solids		Soluble in waste sludge	Effluent		Vent gas
Reactor No.	Date	Elapsed time (hr)	Volume (L)	Phenol (mg/L)	Phenol mass (mg)	Phenol loading (ug/g MLSS)	Phenol mass (mg)	Phenol mass (mg)	Phenol (mg/L)	Phenol mass (mg)	Phenol mass (mg)
1	06/01/83	25.25	10.6	981	10,400	94.1	0.44	<0.43	<0.50	<5.3	<1.49
1	06/02/83	21.00	3.91	999	3,900	41.7	0.19	<0.15	<0.50	<2.0	
1	06/03/83	27.00	16.3	1051	17,100				<0.50	<8.2	<1.60
1	06/04/83	24.00	9.46	974	9,200			<0.18	<0.50	<4.7	<1.42
1	06/05/83	26.00	8.21	998	8,200			<0.20	<0.50	<4.1	<1.54
1	06/06/83	21.00	11.0	962	10,600			<0.15			
1	06/07/83	24.00	5.35	957	5,100			<0.15			
1	06/08/83	24.00	10.6	965	10,200	90	1.41		<0.50	<5.3	<1.90
2	06/01/83	25.25	10.4	981	10,200			<0.45	<0.50	<5.2	
2	06/02/83	21.00	5.28	999	5,300	32.7	0.14	<0.15	<0.50	<2.6	<1.25
2	06/03/83	27.00	16.1	1051	16,900				<0.50	<8.0	<1.60
2	06/04/83	24.00	7.98	974	7,800				<0.50	<4.0	<1.42
2	06/05/83	26.00	9.24	998	9,200				<0.50	<4.5	<1.76
2	06/06/83	21.00	9.39	962	9,000			<0.15			
2	06/07/83	24.00	10.4	957	10,000			<0.05			
2	06/08/83	24.00	12.6	965	12,200	62.9	0.32		<0.50	<6.3	<1.90

TABLE 94. MATERIAL BALANCE SUMMARY FOR LAS UNITS PHENOL ^{14}C ANALYSIS

Reactor No.	Date	Elapsed time (hr)	Feed			Waste solids		Soluble in waste sludge	Soluble effluent		Vent gas
			Volume (L)	^{14}C (MM DPM/L)	Total ^{14}C (MM DPM)	^{14}C (DPM/mg MLSS)	Total ^{14}C (MM DPM)	Total ^{14}C (MM DPM)	^{14}C (MM DPM/L)	Total ^{14}C (MM DPM)	Total ^{14}C (MM DPM)
1	06/01/83	25.25	10.6	0.970	10.3			0.04	0.048	0.51	4.78
1	06/02/83	21.00	3.91	0.990	3.87			0.03	0.100	0.39	2.44
1	06/03/83	27.00	16.3	1.032	16.9			0.00	0.191	3.12	6.77
1	06/04/83	24.00	9.46	1.144	10.8			0.07	0.197	1.86	5.09
1	06/05/83	26.00	8.21	1.189	9.76	274	1.57	0.08	0.206	1.69	7.23
1	06/06/83	21.00	11.0	1.233	13.6			0.06	0.206	2.27	6.40
1	06/07/83	24.00	5.35	1.450	7.76			0.06	0.189	1.01	6.59
1	06/08/83	24.00	10.6	1.474	15.6	316	2.35	0.00	0.149	1.57	9.61
2	06/01/83	25.25	10.4	0.970	10.0			0.03	0.037	0.38	8.66
2	06/02/83	21.00	5.28	0.990	5.23			0.04	0.116	0.61	4.67
2	06/03/83	27.00	16.1	1.032	16.6			0.00	0.172	2.77	2.74
2	06/04/83	24.00	7.98	1.144	9.13			0.00	0.185	1.48	5.41
2	06/05/83	26.00	9.24	1.189	11.0	197	1.29	0.00	0.208	1.92	6.32
2	06/06/83	21.00	9.39	1.233	11.6			0.06	0.206	1.94	6.87
2	06/07/83	24.00	10.4	1.450	15.1			0.02	0.190	1.98	9.19
2	06/08/83	24.00	12.6	1.474	18.5	350	1.76	0.00	0.136	1.71	10.4

tional analysis. However, analysis of the ^{14}C data indicates that toluene is more readily biodegraded than either phenol or aniline as indicated by the high percentage of feed ^{14}C recovered as $^{14}CO_2$ in the bubblers and the low percentage of ^{14}C recovered as a soluble metabolite. Analysis of bubbler trains for reactors 1 and 2 revealed that less than 0.60 and 0.33%, respectively, of the ^{14}C collected could be attributed to toluene.

Operating summaries and material balances for the toluene study are presented in Figs. 56 and 57 and Tables 95 and 96. Toluene concentrations in the vent gas, effluent, and grab samples of the mixed liquor were found to be highly variable from day to day. Concentrations of toluene ranging from <0.75 ppm_v to 166 ppm_v and <0.75 ppm_v to 190 ppm_v were measured for reactors 1 and 2, respectively. Correlation of the daily concentrations of toluene in the vent gas and in the mixed liquor is discussed later in this section.

Material balances indicate that 11.6 and 11.9% of the toluene fed to reactors 1 and 2, respectively, was stripped in the off-gas. However, the average values are relatively high due to high concentrations of toluene detected in the vent gas on days 6-28, 6-29, and 6-30. These high concentrations occurred immediately following a period of low feed flows as shown in Figs. 56 and 57. The high vent gas concentrations measured during the period 6-28 to 6-30 may also be correlated with a decrease in MLSS and high effluent suspended solids during the same period.

Conversely, during periods of relatively steady-state operation stripping of toluene was minimal. The toluene concentration in the vent gas was below the detection limit of 0.75 ppm_v for approximately 30% of the samples from each reactor. At a vent gas concentration of 0.75 ppm_v, less than 0.4% of the toluene in the feed was stripped from the mixed liquor. This demonstrates that when the biological conversion process is "well-behaved" and free from external transients, toluene stripping is negligible.

Aniline --

Examination of the material balances for the parent compound and ^{14}C reveal that the fate mechanisms for aniline are almost identical to the fate mechanisms for phenol. Aniline is readily biodegraded with over 55% of the feed ^{14}C converted to $^{14}CO_2$. Aniline was never detected in either the effluent or the bubblers at an analytical detection limit of 0.10 mg/L. Instrument changes during the last 3 days of the aniline run resulted in analytical detection limits of 0.01 mg/L. During this period, the average concentration of aniline in the effluent was found to be 0.032 and 0.028 mg/L for reactors 1 and 2, respectively. Average loadings of aniline on biological solids were 30.6 and 33.2 μg/g MLSS for reactors 1 and 2, respectively. Operating summaries and daily material balances for aniline are presented in Figs. 58, 59, and Tables 97 and 98.

Pentachlorophenol --

Pentachlorophenol was chosen as a test compound primarily to study the sorption mechanism since it has a high octanol-water partition coefficient (131,000) in the associated form. As noted earlier, the dissociated form of

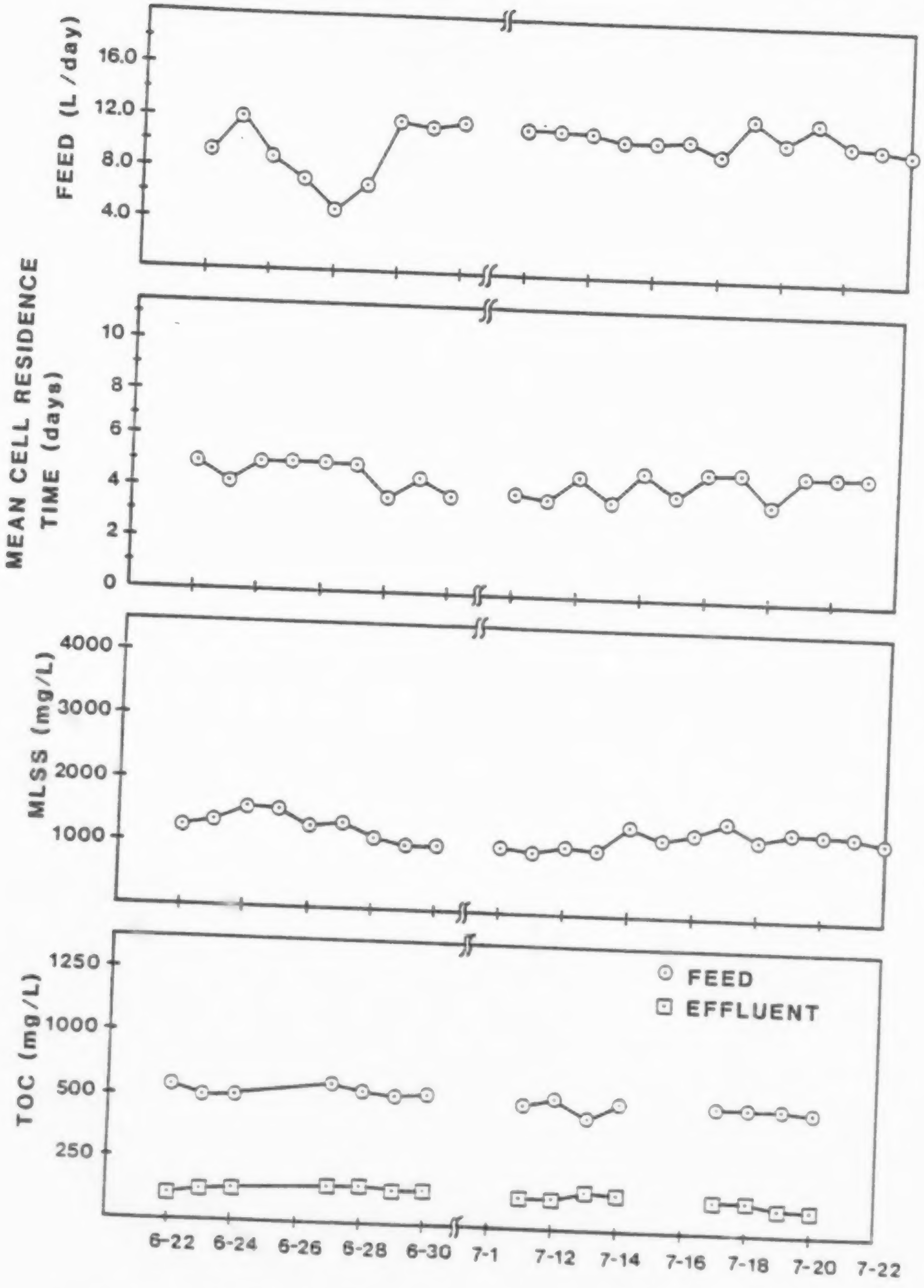

FIG. 56. OPERATING SUMMARY - TOLUENE REACTOR NO. 1

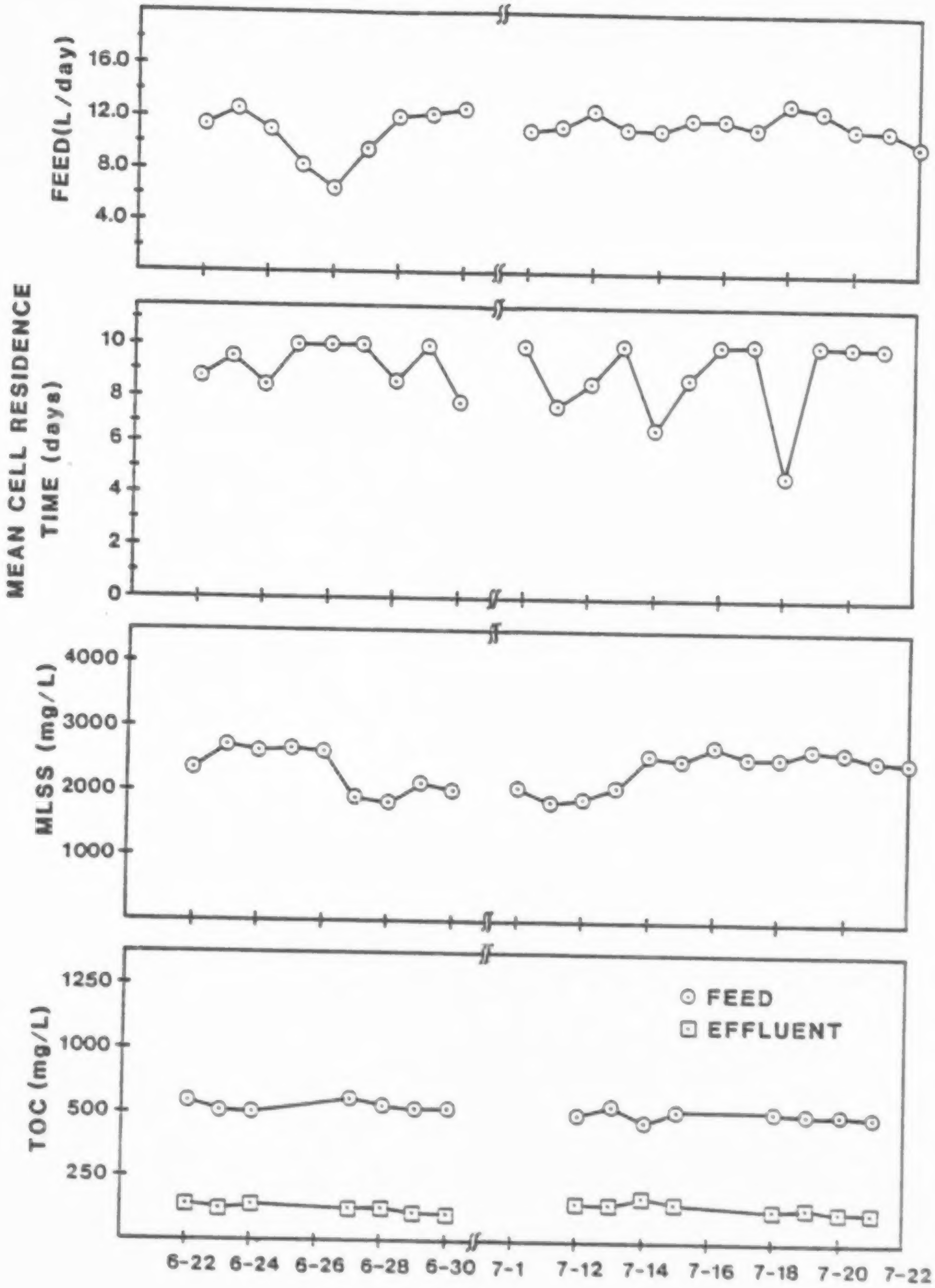

FIG. 57. OPERATING SUMMARY - TOLUENE REACTOR NO. 2

TABLE 95. MATERIAL BALANCE SUMMARY FOR LAS UNITS TOLUENE CONVENTIONAL ANALYSIS

			Feed			Waste Solids		Soluble in waste sludge	Soluble effluent		Vent gas
Reactor No.	Date	Elapsed time (hr)	Volume (L)	Toluene (mg/L)	Toluene mass (mg)	Toluene loading (ug/g MLSS)	Toluene mass (mg)	Toluene mass (mg)	Toluene (mg/L)	Toluene mass (mg)	Toluene mass (mg)
1	06/22/83	23.50	9.15								22.5
1	06/23/83	24.50	12.2					0.00			441
1	06/24/83	24.00	9.77								<8.98
1	06/25/83	31.50	9.29								
1	06/26/83	24.25	4.98								
1	06/27/83	17.25	5.01	153	767						11.8
1	06/28/83	23.50	11.7	240	2800			0.00			2100
1	06/29/83	25.00	12.0	183	2200			0.00	<0.05	<0.60	233
1	06/30/83	23.50	11.7					0.00	0.20	2.34	1230
1	07/01/83	23.50	11.4	176	2000			0.00			<9.35
1	07/11/83	28.50	13.7	236	3230			0.00			50.7
1	07/12/83	23.75	11.3	240	2710			0.00	0.09	1.01	28.7
1	07/13/83	24.25	10.9	230	2510			0.00	<0.05	<0.54	14.3
1	07/14/83	24.00	10.8	177	1910			0.02	<0.05	<0.54	<7.78
1	07/15/83	24.08	11.0	242	2660			0.00	<0.05	<0.55	7.13
1	07/16/83	24.33	10.0	167	1670						16.0
1	07/17/83	23.83	12.8								
1	07/18/83	22.50	10.3	191	1970			0.00	<0.05	<0.51	113
1	07/19/83	23.92	12.6	209	2630						
1	07/20/83	24.50	11.0	150	1650						
1	07/21/83	23.58	10.7	172	1840						<10.04
1	07/22/83	23.00	10.0								< 9.88
2	06/22/83	23.50	11.1								256
2	06/23/83	24.50	12.9					0.00			15.3
2	06/24/83	24.00	11.0					0.00			41.7
2	06/25/83	31.50	11.0								
2	06/26/83	24.25	6.54								
2	06/27/83	17.25	6.95	153	1060						13.7
2	06/28/83	23.50	11.8	240	2830			0.00			2180
2	06/29/83	25.00	12.8	183	2340				0.60	7.66	177
2	06/30/83	23.50	12.9					0.00	0.40	5.17	523
2	07/01/83	23.50	10.8	176	1900			0.07			16.1
2	07/11/83	28.50	13.5	236	3190			0.00			1168
2	07/12/83	23.75	12.4	240	2980			0.00	0.50	6.20	9.15
2	07/13/83	24.25	11.5	230	2650			0.01	<0.05	<0.57	<8.98
2	07/14/83	24.00	11.1	177	1960			0.00	<0.05	<0.56	<8.53
2	07/15/83	24.08	11.9	242	2880			0.00	<0.05	<0.60	<8.78
2	07/16/83	24.33	12.1	167	2020						9.64
2	07/17/83	23.83	11.2								
2	07/18/83	22.50	12.3	191	2350			0.00	0.40	4.93	219
2	07/19/83	23.92	12.6	209	2630						
2	07/20/83	24.50	11.5	150	1730						
2	07/21/83	23.58	11.0	172	1890						<10.04
2	07/22/83	23.00	9.57								< 9.45

TABLE 96. MATERIAL BALANCE SUMMARY FOR LAS UNITS TOLUENE ^{14}C ANALYSIS

Reactor Number	Date	Elapsed time (hr)	Feed			Waste solids		Soluble in waste sludge	Soluble effluent		Vent gas
			Volume (L)	^{14}C (MM DPM/L)	Total ^{14}C (MM DPM)	^{14}C (DPM/mg MLSS)	Total ^{14}C (MM DPM)	Total ^{14}C (MM DPM)	^{14}C (MM DPM/L)	Total ^{14}C (MM DPM)	Total ^{14}C (MM DPM)
1	06/22/83	23.50	9.15	2.09	19.1			0.12	0.031	0.28	12.5
1	06/23/83	24.50	12.2	1.77	21.5			0.00	0.059	0.72	11.1
1	06/24/83	24.00	9.77	1.13	11.1			0.07	0.061	0.60	6.93
1	06/25/83	31.50	9.29								
1	06/26/83	24.25	4.98								
1	06/27/83	17.25	5.01	0.611	3.06	749	2.43	0.02	0.054	0.27	2.86
1	06/28/83	23.50	11.7	0.607	7.09			0.00	0.053	0.62	3.09
1	06/29/83	25.00	12.0	1.01	12.1			0.00	0.069	0.83	7.92
1	06/30/83	23.50	11.7	0.874	10.2			0.00	0.027	0.32	6.46
1	07/01/83	23.50	11.4	0.814	9.27	717	2.58	0.00	0.047	0.53	6.67
1	07/11/83	28.50	13.7					0.00			
1	07/12/83	23.75	11.3	0.193	2.18	380	1.21	0.00	0.017	0.19	2.20
1	07/13/83	24.25	10.9	0.180	1.96	317	1.27	0.00	0.015	0.16	1.84
1	07/14/83	24.00	10.8	0.149	1.61	260	0.933	0.01	0.018	0.20	1.75
1	07/15/83	24.08	11.0	0.905	9.98	507	2.27	0.00	0.032	0.35	7.19
1	07/16/83	24.33	10.1	0.752	7.54	568	1.70	0.01	0.035	0.35	5.65
1	07/17/83	23.83	12.8			534	1.88	0.00			
1	07/18/83	22.50	10.3	0.864	8.89	677	3.37	0.00	0.047	0.48	7.26
1	07/19/83	23.92	12.6	0.830	10.4	705	2.35	0.01	0.041	0.51	6.89
1	07/20/83	24.50	11.0	0.70	0.77	528	1.70		0.024	0.26	1.27
1	07/21/83	23.58	10.7	0.069	0.73	418	1.31	0.00	0.015	0.17	1.00
1	07/22/83	23.00	10.0	0.042	0.42	309			0.016	0.16	0.63
2	06/22/83	23.50	11.1	2.09	23.2			0.21	0.047	0.52	13.6
2	06/23/83	24.50	12.9	1.77	22.8			0.00	0.066	0.85	10.1
2	06/24/83	24.00	11.0	1.13	12.4			0.00	0.088	0.97	9.46
2	06/25/83	31.50	11.0								
2	06/26/83	24.25	6.54								
2	06/27/83	17.25	6.95	0.611	4.24	550	1.15	0.20	0.072	0.50	5.05
2	06/28/83	23.50	11.8	0.607	7.14			0.00	0.039	0.46	4.56
2	06/29/83	25.00	12.8	1.007	12.9				0.056	0.71	8.97
2	06/30/83	23.50	12.9	0.874	11.3			0.00	0.058	0.75	8.34
2	07/01/83	23.50	10.8	0.814	8.75	619	1.55	0.04	0.053	0.57	5.25
2	07/11/83	28.50	13.5					0.00			
2	07/12/83	23.75	12.4	0.193	2.40	413	1.12	0.00	0.027	0.33	2.75
2	07/13/83	24.25	11.5	0.180	2.07	335	0.775	0.06	0.019	0.22	2.68
2	07/14/83	24.00	11.1	0.149	1.65	281	1.36	0.00	0.023	0.25	1.99
2	07/15/83	24.08	11.9	0.905	10.7	342	1.21	0.00	0.045	0.53	6.45
2	07/16/83	24.33	12.1	0.752	9.09	359	1.20	0.16	0.043	0.52	7.14
2	07/17/83	23.83	11.2			421	1.19				
2	07/18/83	22.50	12.3	0.864	10.7	473	3.40	0.00	0.046	0.57	7.61
2	07/19/83	23.92	12.6	0.830	10.4	507	1.55	0.21	0.048	0.60	7.34
2	07/20/83	24.50	11.5	0.070	0.81	404	1.24		0.030	0.34	2.23
2	07/21/83	23.58	11.0	0.069	0.75	332	0.958	0.11	0.020	0.22	1.52
2	07/22/83	23.00	9.6	0.042	0.40	262			0.017	0.16	1.85

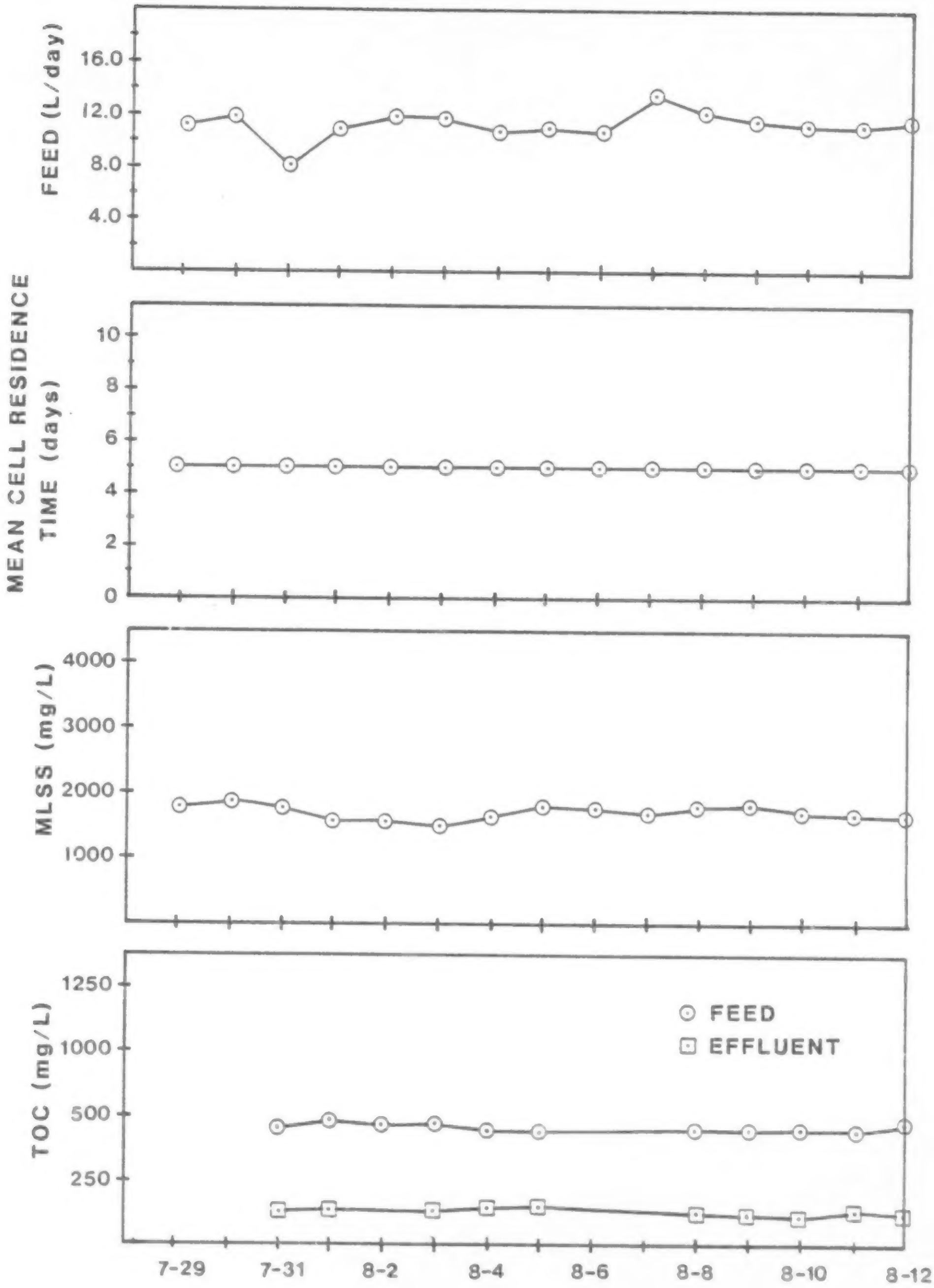

FIG. 58. OPERATING SUMMARY - ANILINE REACTOR NO. 1

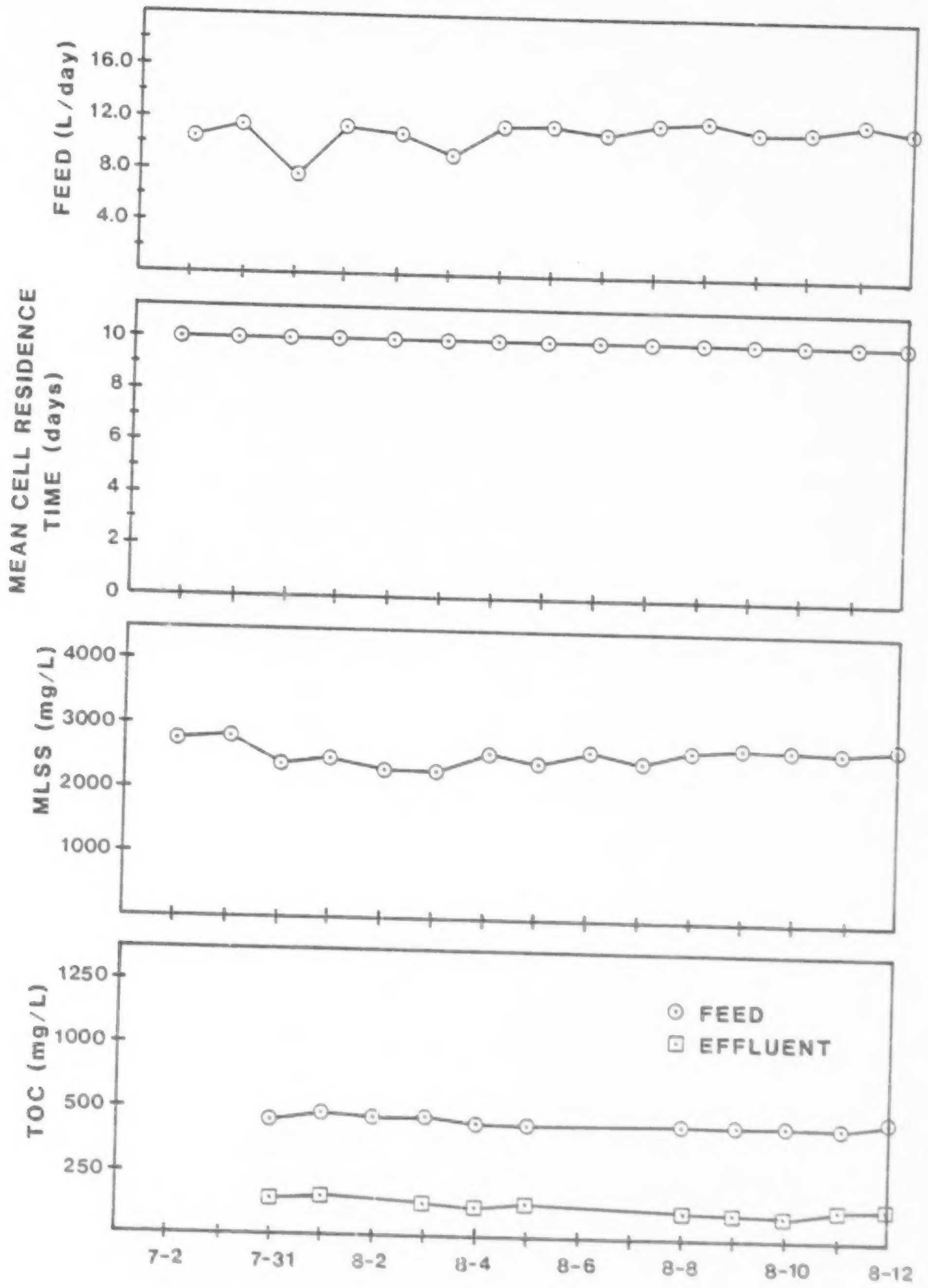

FIG. 59. OPERATING SUMMARY - ANILINE REACTOR NO. 2

TABLE 97. MATERIAL BALANCE SUMMARY FOR LAS UNITS ANILINE CONVENTIONAL ANALYSIS

Reactor No.	Date	Elapsed time (hr)	Feed			Waste Solids		Soluble in waste sludge	Effluent		Vent gas
			Volume (L)	Aniline (mg/L)	Aniline mass (mg)	Aniline loading (ug/g MLSS)	Aniline mass (mg)	Aniline mass (mg)	Aniline (mg/L)	Aniline mass (mg)	Aniline mass (mg)
1	07/29/83	24.00	11.1					<0.06	0.150	1.66	<0.46
1	07/30/83	30.00	14.6					<0.04	<0.100	<1.46	
1	07/31/83	20.50	6.94					≤0.04	<0.100	<0.69	
1	08/01/83	22.50	10.2	130	1330			<0.03	<0.100	<1.02	<0.43
1	08/02/83	21.00	10.4	120	1250			<0.08	<0.100	<1.04	<0.44
1	08/03/83	22.75	11.1	120	1332			<0.04	<0.100	<1.11	<0.40
1	08/04/83	22.50	9.95	105	1040			<0.03	<0.100	<1.00	<0.43
1	08/05/83	24.00	10.9	104	1130	<51.2	0.214	<0.10	<0.100	<1.09	<0.46
1	08/06/83	20.75	9.38					<0.04			
1	08/07/83	24.25	13.7					<0.05			
1	08/08/83	27.25	14.1	105	1480	<27.9	0.111	<0.03	<0.100	<1.41	<0.52
1	08/09/83	23.75	11.4	100	1140			<0.00	<0.010	<0.11	<0.45
1	08/10/83	24.25	11.4	100	1140	22.5	0.084	0.00	<0.010	<0.11	<0.47
1	08/11/83	23.50	10.8	97	1050	22.6	0.085	0.00	0.014	0.15	<0.45
1	08/12/83	22.00	10.5	93	977	28.9	0.104		0.051	0.53	<0.45
2	07/29/83	24.00	10.5					<0.06	0.100	1.05	<0.46
2	07/30/83	30.00	14.8					<0.03	<0.100	<1.48	
2	07/31/83	20.50	6.56					<0.02	<0.100	<0.66	
2	08/01/83	22.50	10.6	130	1380			<0.02	<0.100	<1.06	<0.43
2	08/02/83	21.00	9.48	120	1140			<0.07	<0.100	<0.95	<0.44
2	08/03/83	22.75	8.81	120	1060			<0.02	<0.100	<0.88	<0.40
2	08/04/83	22.50	10.8	105	1134			<0.03	<0.100	<1.08	<0.43
2	08/05/83	24.00	11.7	104	1220	< 36.7	0.103	<0.04	<0.100	<1.17	<0.46
2	08/06/83	20.75	9.54					<0.04			
2	08/07/83	24.25	11.9					<0.03			
2	08/08/83	27.25	13.6	105	1430	< 33.4	0.101	<0.02	<0.100	<1.36	<0.52
2	08/09/83	23.75	11.2	100	1110			0.00	<0.010	<0.11	<0.45
2	08/10/83	24.25	11.5	100	1150	28.5	0.086	0.00	<0.010	<0.12	<0.47
2	08/11/83	23.50	11.9	97	1150	30.7	0.092	0.00	<0.010	<0.12	<0.45
2	08/12/83	22.00	10.6	93	986	36.8	0.116		0.069	0.73	<0.45

TABLE 98. MATERIAL BALANCE SUMMARY FOR LAS UNITS ANILINE ^{14}C ANALYSIS

Reactor No.	Date	Elapsed time (hr)	Feed: Volume (L)	Feed: ^{14}C (MM DPM/L)	Feed: Total ^{14}C (MM DPM)	Waste solids: ^{14}C (DPM/mg MLSS)	Waste solids: Total ^{14}C (MM DPM)	Soluble in waste sludge: Total ^{14}C (MM DPM)	Soluble effluent: ^{14}C (MM DPM/L)	Soluble effluent: Total ^{14}C (MM DPM)	Vent gas: Total ^{14}C (MM DPM)
1	07/29/83	24.00	11.1	0.757	8.39			0.02	0.034	0.38	4.17
1	07/30/83	30.00	14.6								
1	07/31/83	20.50	6.94								
1	08/01/83	22.50	10.2	0.919	9.33	496	1.71	0.03	0.121	1.23	4.89
1	08/02/83	21.00	10.4	0.939	9.74	562	1.95	0.11	0.132	1.37	5.08
1	08/03/83	22.75	11.1	0.917	10.2	621	2.06	0.05	0.139	1.54	5.21
1	08/04/83	22.50	9.95	0.773	7.69	606	2.20	0.04	0.138	1.37	4.37
1	08/05/83	24.00	10.9	0.765	8.34	600	2.51	0.14	0.139	1.51	5.14
1	08/06/83	20.75	9.38								
1	08/07/83	24.25	13.7								
1	08/08/83	27.25	14.1	0.753	10.62	686	2.73	0.03	0.107	1.51	6.17
1	08/09/83	23.75	11.4	0.744	8.50	657	2.68	0.02	0.118	1.35	5.36
1	08/10/83	24.25	11.4	0.756	8.61	669	2.55	0.03	0.100	1.14	5.23
1	08/11/83	23.50	10.9	0.748	8.13	700	2.62	0.03	0.108	1.17	4.65
1	08/12/83	22.00	10.5	0.809	8.48	711	2.57		0.111	1.16	4.17
2	07/29/83	24.00	10.5	0.757	7.95			0.02	0.040	0.42	4.13
2	07/30/83	30.00	14.8								
2	07/31/83	20.50	6.56								
2	08/01/83	22.50	10.6	0.919	9.75	314	0.87	0.02	0.106	1.12	5.13
2	08/02/83	21.00	9.48	0.937	8.91	381	0.95	0.07	0.100	0.95	4.73
2	08/03/83	22.75	8.81	0.917	8.08	423	1.08	0.02	0.109	0.96	4.49
2	08/04/83	22.50	10.8	0.773	8.37	440	1.26	0.03	0.111	1.20	4.94
2	08/05/83	24.00	11.7	0.765	8.92	493	1.38	0.04	0.110	1.28	5.60
2	08/06/83	20.75	9.54								
2	08/07/83	24.25	11.9								
2	08/08/83	27.25	13.6	0.753	10.3	541	1.64	0.02	0.100	1.36	6.25
2	08/09/83	23.75	11.2	0.744	8.29	528	1.62	0.03	0.107	1.19	5.06
2	08/10/83	24.25	11.5	0.756	8.70	547	1.66	0.01	0.096	1.10	5.40
2	08/11/83	23.50	11.9	0.747	8.90	595	1.78	0.01	0.100	1.19	5.35
2	08/12/83	22.00	10.6	0.810	8.56	563	1.78		0.110	1.16	4.32

pentachlorophenate has a K_{ow} of 7,700 at pH 7 in pure water systems. These experiments were buffered close to pH 7. Minimal acclimation time was allowed prior to system startup with pentachlorophenol so that the sorption mechanism might be better tested in the LAS runs.

Pentachlorophenol was found to be completely resistant to bio-oxidation under the test conditions in the LAS reactors. Tables 91 and 92 indicate 102 to 104% recovery of the parent pentachlorophenol and 93 to 97% recovery of the ^{14}C.

Sorption onto biological solids was the major pathway for the removal of pentachlorophenol from both LAS systems. Both the test material balance and the ^{14}C balance indicate that approximately 6 to 8% of the pentachlorophenol fed to the system is lost via sorption to waste solids. Operating summaries and daily material balances for the pentachlorophenol study are presented in Figs. 60 and 61 and Tables 99 and 100. Data in Table 99 indicate that pentachlorophenol loadings on biological solids range from 1210 to 2180 μg/g for reactor 1 and from 943 to 2590 μg/g for reactor 2.

Validation of Stripping Predictive Fate Method (PFM)

The PFM which was developed for the stripping rate constant is as follows:

$$K_a{}^{st}a = \frac{Q_{air}}{V} \cdot 3.71 \times 10^{-3} H_c{}^{1.045} \qquad \text{Eqn. 62}$$

where:

$K_a{}^{st}a$ = stripping rate constant (hr^{-1}),
Q_{air} = air flow (L/min),
H_c = Henry's law constant (torr • L/g-mole), and
V = reactor volume (L).

Assuming first order kinetics, the vent gas concentration can be predicted from the following equation:

$$C_v = \frac{K_a{}^{st}a \cdot C_a \cdot V \cdot \frac{1g}{1000\ mg} \cdot \frac{1g\text{-mole}}{MW\ g} \cdot \frac{22.4\ L}{g\text{-mole}} \cdot \frac{10^6\ ppm_v}{L\ toluene/L\ air}}{Q_{air} \cdot \frac{60\ min}{hr}} \qquad \text{Eqn. 63}$$

Simplifying,

$$C_v = \frac{K_a{}^{st}a \cdot C_a \cdot V \cdot 373.3}{Q_a \cdot MW} \qquad \text{Eqn. 64}$$

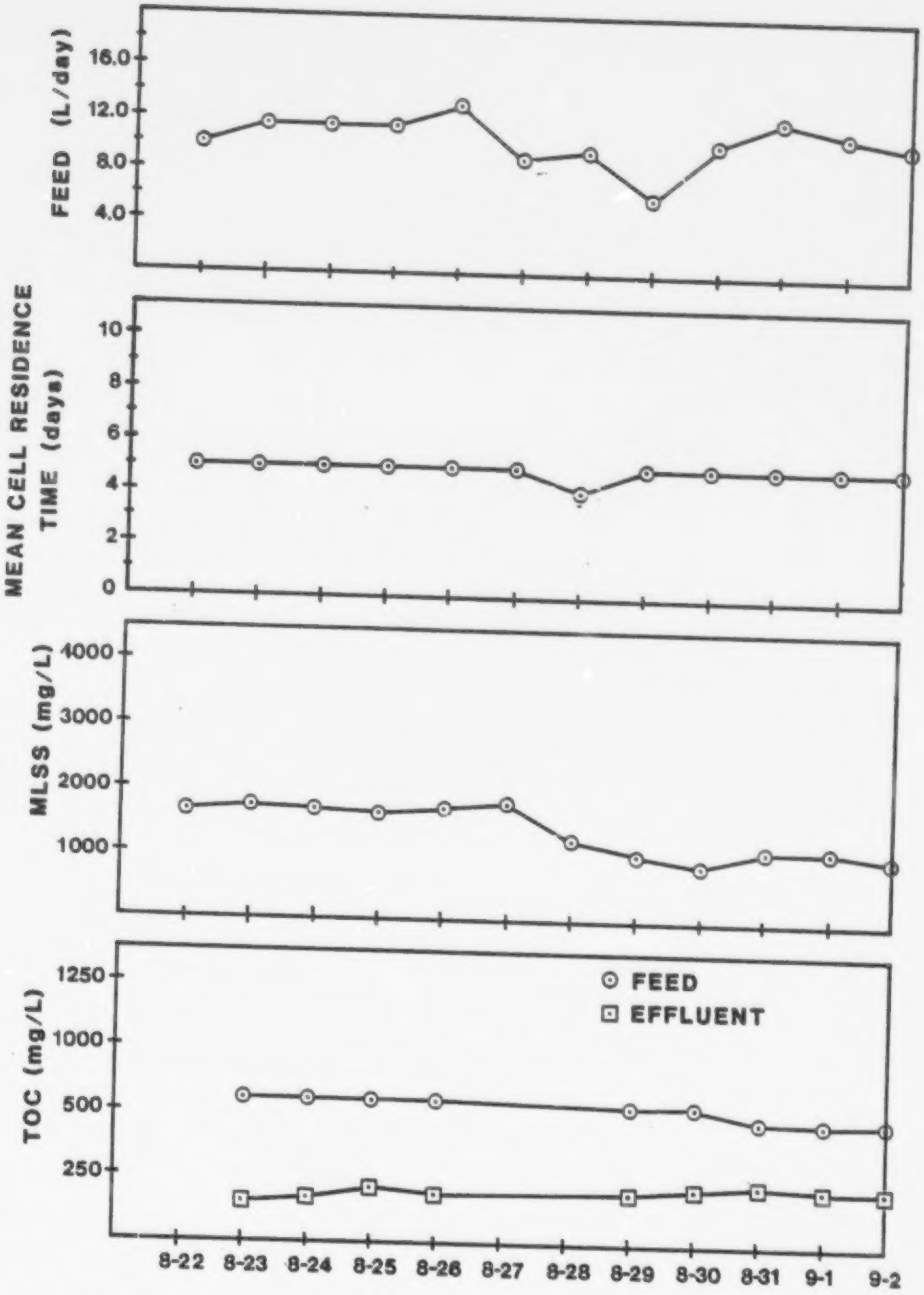

FIG. 60. OPERATING SUMMARY - PENTACHLOROPHENOL REACTOR NO. 1

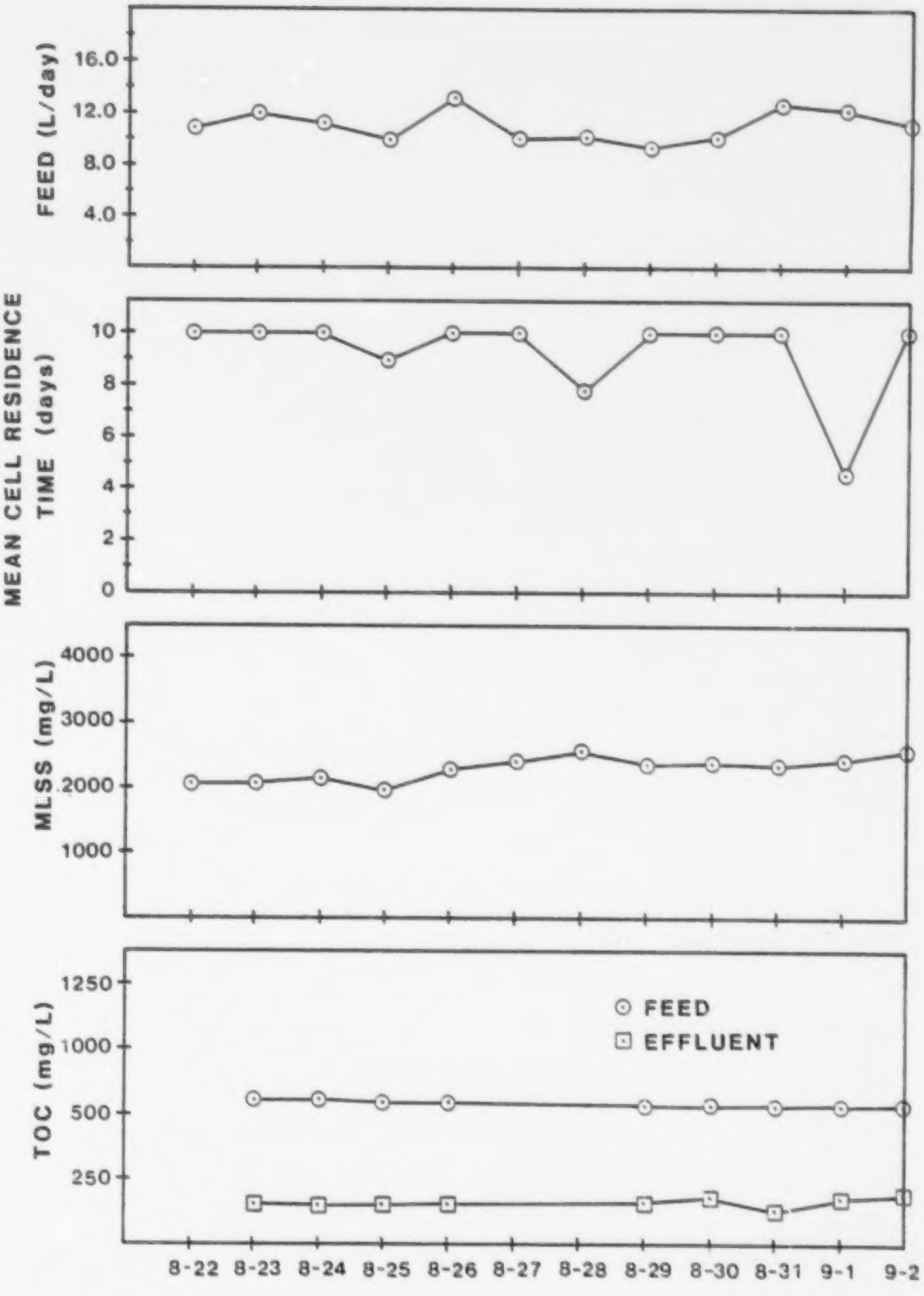

FIG. 61. OPERATING SUMMARY - PENTACHLOROPHENOL REACTOR NO. 2

TABLE 99. MATERIAL BALANCE SUMMARY FOR LAS UNITS PCP CONVENTIONAL ANALYSIS

Reactor No.	Date	Elapsed time (hr)	Feed			Waste Solids		Soluble in waste sludge	Effluent		Vent gas
			Volume (L)	PCP (mg/L)	PCP mass (mg)	PCP loading (ug/g MLSS)	PCP mass (mg)	PCP mass (mg)	PCP (mg/L)	PCP mass (mg)	PCP mass (mg)
1	08/22/83	18.42	7.59	10.0	75.9	1440	5.33	6.82	6.2	47.1	<0.20
1	08/23/83	24.33	11.7	11.3	132	1530	6.09	8.28	9.2	108	
1	08/24/83	23.15	11.0	11.3	124	2430	9.35	1.94	10.8	119	
1	08/25/83	23.75	11.3	9.8	111	2320	9.95	1.96	9.8	111	
1	08/26/83	21.00	11.4	7.8	88.8	2360	9.41	3.89	7.2	82.0	
1	08/27/83	24.25	9.05	8.0	72.4			4.73	7.4	67.0	
1	08/28/83	26.92	10.8	7.4	79.8	1230	5.23	0.00	8.0	86.3	
1	08/29/83	23.83	5.94	8.0	47.5	1260	2.99	1.02	7.3	43.4	
1	08/30/83	23.00	9.71	7.6	73.8	1210	2.52	0.88	6.8	66.0	
1	08/31/83	21.75	10.8	8.4	90.8	2270	6.11		6.6	71.3	
1	09/01/83	26.75	12.8	6.5	79.1	3180	8.74		6.2	75.5	
1	09/02/83	24.00	10.0	7.0	70.2	2410	5.76		6.2	62.2	
2	08/22/83	18.42	8.28	10.0	82.8	1080	2.49	2.59	5.4	44.7	<0.20
2	08/23/83	24.33	12.0	11.3	136	1240	2.93	2.94	9.8	118	
2	08/24/83	23.15	11.0	11.3	124	2590	6.26	1.66	10.4	114	
2	08/25/83	23.75	9.75	9.8	95.6	2460	6.73	0.00	10.1	98.5	
2	08/26/83	21.00	11.4	7.8	89.2	2420	6.63	4.32	8.0	91.5	
2	08/27/83	24.25	10.1	8.0	80.8			2.29	7.4	74.7	
2	08/28/83	26.92	11.3	7.4	83.3	1530	6.39	0.00	7.4	83.3	
2	08/29/83	23.83	9.27	8.0	74.2	1130	3.06	1.30	7.6	70.5	
2	08/30/83	23.00	9.58	7.6	72.8	943	2.59	1.03	7.9	75.7	
2	08/31/83	21.75	11.4	8.4	96.1	2350	6.26		8.8	101	
2	09/01/83	26.75	13.5	6.5	87.6	2440	18.5		7.1	95.7	
2	09/02/83	24.00	11.1	7.0	77.6	1630	4.84		6.6	73.1	

TABLE 100. MATERIAL BALANCE SUMMARY FOR LAS UNITS PCP ^{14}C ANALYSIS

Reactor No.	Date	Elapsed time (hr)	Feed			Waste solids		Soluble in waste sludge	Soluble effluent		Vent gas
			Volume (L)	^{14}C (MM DPM/L)	Total ^{14}C (MM DPM)	^{14}C (DPM/mg MLSS)	Total ^{14}C (MM DPM)	Total ^{14}C (MM DPM)	^{14}C (MM DPM/L)	Total ^{14}C (MM DPM)	Total ^{14}C (MM DPM)
1	08/22/83	18.42	7.59	1.19	9.05	121	0.448	0.193	0.175	1.33	0.03
1	08/23/83	24.33	11.7	1.21	14.2	205	0.815	0.531	0.590	6.93	
1	08/24/83	23.15	11.0	1.11	12.2	262	1.01	0.137	0.762	8.41	0.04
1	08/25/83	23.75	11.3	1.15	13.0	303	1.301	0.164	0.821	9.30	
1	08/26/83	21.00	11.4	1.10	12.5	237	0.942	0.433	0.865	9.85	0.06
1	08/27/83	24.25	9.05	1.12	10.1	176	0.773	0.577	0.902	8.16	
1	08/28/83	26.92	10.8	0.952	10.3	183	0.780	0.000	0.863	9.31	
1	08/29/83	23.83	5.94	0.988	5.87	341	0.805	0.119	0.850	5.05	0.08
1	08/30/83	23.00	9.71	0.944	9.17	302	0.631	0.103	0.789	7.66	
1	08/31/83	21.75	10.8	0.851	9.20	317	0.852		0.797	8.62	0.06
1	09/01/83	26.75	12.8	0.831	10.1	279	0.767		0.714	8.69	
1	09/02/83	24.00	10.0	0.842	8.45	222	0.532		0.735	7.37	0.04
2	08/22/83	18.42	8.28	1.19	9.87	114	0.261	0.086	0.180	1.49	0.07
2	08/23/83	24.33	12.0	1.21	14.5	173	0.409	0.205	0.683	8.20	
2	08/24/83	23.15	11.0	1.11	12.2	255	0.618	0.134	0.835	9.15	0.07
2	08/25/83	23.75	9.75	1.15	11.2	273	0.746	0.000	0.852	8.31	
2	08/26/83	21.00	11.4	1.10	12.6	216	0.592	0.457	0.914	10.5	0.08
2	08/27/83	24.25	10.1	1.12	11.3	150	0.428	0.287	0.926	9.35	
2	08/28/83	26.92	11.30	0.952	10.7	187	0.778	0.000	0.908	10.2	
2	08/29/83	23.83	9.27	0.988	9.15	251	0.678	0.160	0.944	8.75	0.10
2	08/30/83	23.00	9.58	0.944	9.05	233	0.640	0.113	0.866	8.30	
2	08/31/83	21.75	11.4	0.851	9.73	221	0.588		0.828	9.47	0.09
2	09/01/83	26.75	13.5	0.831	11.2	216	1.64		0.740	9.97	
2	09/02/83	24.00	11.1	0.823	9.12	182	0.540		0.755	8.38	0.12

where:

C_v = concentration in the vent gas (ppm_v),
C_a = soluble concentration in the mixed liquor (mg/L), and
MW = molecular weight of compound (g/g-mole).

Equation 64 was used to predict the vent gas concentrations from the LAS reactors for the toluene study. The analytical detection limit for the experimental system was 0.05 mg/L for the aqueous phase. At these detection limits, a maximum vent gas concentration of 2.97 ppm_v would be calculated using Eqn. 64. The results of this analysis are shown in Table 101. Nine data points were available for which the soluble toluene concentration in the mixed liquor was above the detectable limit. Five of the predicted vent gas concentrations were within ±50% of the measured value. Of the twelve data points for which C_a was below the detectable limit, nine of the measured vent gas concentrations were below the maximum value predicted by Eqn. 64.

Daily average data on liquid and gas concentrations were used to make this comparison. When a similar comparison is made selectively comparing data when liquid and vent gas samples were taken simultaneously, the prediction improves into the ±25% range. Both approaches are important since limited data presently exist where simultaneous liquid and gas concentrations are known.

Stripping losses during LAS studies for phenol, aniline, and pentachlorophenol were negligible, as shown in Table 102. Stripping losses for these studies were estimated in order to verify the stripping PFM for low volatility compounds. These calculations were performed using average values of air flow rates and soluble mixed liquor concentrations measured during the studies with each compound. The stripping PFM was also used to project the percentage of the feed compound which could be stripped if no other removal mechanism was active, i.e., no sorption or bio-oxidation. This analysis for a continuously fed complete-mixed reactor takes the form:

$$C_a = \frac{C_{ao}}{1 + K_a^{st}a\ (HRT)} \qquad \text{Eqn. 65}$$

where:

C_a = aqueous phase effluent concentration (mg/L),
C_{ao} = feed concentration (mg/L),
HRT = hydraulic residence time (hr), and
$K_a^{st}a$ = stripping rate constant (hr^{-1}).

Results of these analyses are presented in Table 102. Estimated and measured removals by stripping are in agreement for both phenol and aniline; however, measured quantities of pentachlorophenol were significantly less than predicted by the model. A possible explanation for the anomaly is

TABLE 101. VALIDATION OF STRIPPING PFM FOR TOLUENE BASED ON DAILY AVERAGE DATA

			Toluene		
Date	Reactor	Air flow (L/min)	Off gas (ppm_v)	Mixed liquor (mg/L)	Predicted off gas (ppm_v)
06/24/83	1	2.02	<0.75	<0.05	<2.97
06/28/83	1	2.18	166.	4.30	256.
06/29/83	1	2.22	17.0	<0.05	<2.97
06/30/83	1	2.15	99.0	1.40	83.2
07/12/83	1	1.88	2.60	0.06	3.56
07/13/83	1	1.70	1.40	<0.05	<2.97
07/14/83	1	1.75	<0.75	<0.05	<2.97
07/15/83	1	1.60	<0.75	<0.05	<2.97
07/18/83	1	1.85	11.0	0.20	11.9
07/21/83	1	2.30	<0.75	<0.05	<2.97
06/24/83	2	2.20	3.20	<0.05	<2.97
06/28/83	2	1.98	190.	4.70	279.
06/29/83	2	2.05	14.0	<0.05	<2.97
06/30/83	2	1.96	46.0	0.80	47.6
07/11/83	2	2.00	83.0	1.30	77.3
07/12/83	2	2.08	<0.75	<0.05	<2.97
07/13/83	2	2.00	<0.75	<0.05	<2.97
07/14/83	2	1.92	<0.75	<0.05	<2.97
07/15/83	2	1.97	<0.75	1.10	65.4
07/18/83	2	1.88	21.0	0.30	17.8
07/21/83	2	2.30	0.75	<0.05	<2.97

TABLE 102. ESTIMATION OF STRIPPING LOSSES FOR LAS SYSTEMS

Compound	Reactor	Predicted K_s (hr^{-1})	Percent of feed: Actual measured	Percent of feed: Estimated based on measured mixed liquor concentrations	Percent of feed: Estimated for no bio-oxidation or sorption losses
Phenol	1	1.98×10^{-4}	<0.017	<0.0003	0.55
	2	1.93×10^{-4}	<0.016	<0.0003	0.50
Aniline	1	5.26×10^{-4}	<0.04	<0.001	0.44
	2	5.16×10^{-4}	<0.04	<0.001	0.44
Pentachlorophenol	1	1.47×10^{-3}	<0.23	3.21	3.60
	2	1.48×10^{-3}	<0.22	3.02	3.43

that pentachlorophenol is predominately in the form of pentachlorophenate at a pH of 7.0. No Henry's law constant was available for pentachlorophenate. However, because the water solubility of pentachlorophenate is much higher than PCP and because the H_c is related inversely with water solubility (Eqns. 25 and 26), it is likely to be lower than the H_c for pentachlorophenol, thus explaining the low recovery of the compound in the vent gas.

Validation of Sorption PFM

The sorption PFM which has been developed incorporates both the rate of sorption and the equilibrium partitioning between solid and liquid phases. First stage sorption rate coefficients were shown to be relatively high in the batch studies (discussed in Section 8), with first order initial sorption rate coefficients of over 75.6, 6.95, and 41.3 hr^{-1} determined for phenol, pentachlorophenol, and 1,4-dichlorobenzene, respectively. Sorption rate constants may be significantly higher than these values, with the maximum rate constant measurable being more a function of the minimum time required to process the sample (4 to 10 min) than a physical property of the chemical. Attainment of equilibrium at low concentrations has been reported by other investigators to take less than 30 min.[6,18]

A relationship that has been used to describe the equilibrium partitioning between sludge solids and an aqueous phase is:

$$C_s = K_{ba} \cdot C_{ae} \qquad \text{Eqn. 66}$$

where:

C_s = equilibrium loading on the sludge solids (μg/g),

C_{ae} = equilibrium aqueous phase concentration (μg/L), and

K_{ba} = sorption distribution coefficient (L/g solids).

This equation is similar to the empirical Freundlich adsorption isotherm equation:

$$C_s = K_f \cdot C_{ae}^{1/n} \qquad \text{Eqn. 67}$$

where:

n, K_f = empirical coefficients.

For values of n near unity, Eqn. 67 is identical to Eqn. 66 and K_f is equivalent to K_{ba}. For values of $n > 1$, a commonplace condition in sorption phenomena, the increase in C_s diminishes with an increase in C_{ae} as C_{ae} gets larger. Although it never reaches an asymptote, the effect is a marked departure from the linear isotherm, and the shape of the curve takes on the appearance of a saturation effect. In environmental science, this effect is called a "non-linear" sorption regime.

Another commonly used adsorption isotherm equation, the Langmuir equation, is based on the assumption that the solid phase has a finite capacity, i.e., a maximum saturation loading which will not be exceeded. The Langmuir isotherm has the form:

$$C_s = \frac{a \cdot b \cdot C_{ae}}{1 + b\, C_{ae}} \qquad \text{Eqn. 68}$$

where:

a, b = empirical constants.

This curve moves from linear sorption behavior to a constant maximum loading through a transition zone. It can be shown that as C_{ae} becomes large, a is equivalent to the maximum loading. As C_{ae} approaches zero, the numerator of Eqn. 68 approaches 1 and Eqn. 68 is equivalent to Eqn. 66 with

$$a \cdot b = K_{ba} \qquad \text{Eqn. 69}$$

An isotherm equation has been proposed in this work of the form:

$$C_s = \frac{K_{ow} \cdot f_L \cdot C_{ae}}{\rho_L} \qquad \text{Eqn. 70}$$

where:

K_{ow} = octanol-water partition coefficient (wt/vol concentration ratio between octanol and water),

f_L = lipid weight fraction of biological solids (μg lipid/μg dry solids), and

ρ_L = density of lipids (g/L).

This equation is based on the premise that the substrate partitions linearly with the lipid fraction of the biomass similarly to a simple extraction process.

Equation 70 is equivalent to Eqn. 66, where

$$K_{ba} = \frac{K_{ow}\, f_L}{\rho_L} \qquad \text{Eqn. 71}$$

A summary of the sorption data collected during the LAS study is shown in Table 103. The average loading for each of the compounds follows the trend of increasing with increasing K_{ow} value. However, aniline shows a higher distribution coefficient than pentachlorophenol, although the K_{ow} value for pentachlorophenol is 5 orders of magnitude greater than for ani-

TABLE 103. SUMMARY OF SLUDGE LOADING DATA FOR LAS STUDY

Compound	Date	Reactor No. 1 Sludge loading (µg/g)	Reactor No. 1 Soluble concentration in mixed liquor (µg/L)	Reactor No. 1 Distribution coefficient (L/g)	Reactor No. 2 Sludge loading (µg/g)	Reactor No. 2 Soluble concentration in mixed liquor (µg/L)	Reactor No. 2 Distribution coefficient (L/g)
Phenol	06/01/83	<94.1	<500				
	06/02/83	<41.7	<500		<32.7	<500	
	06/08/83	<189.7	<500		<62.9	<500	
Aniline	08/08/83	<51.2	<100		<36.7	<100	
	08/08/83	<27.9	<100		<33.4	<100	
	08/10/83	22.5	32	0.70	28.5	19	1.50
	08/11/83	22.6	23	0.98	30.7	30	1.02
	08/12/83	28.9	51	0.57	36.8	104	0.35
Pentachlorophenol	08/22/83	1440	7700	0.19	1080	7000	0.15
	08/23/83	1530	9500	0.16	1240	9800	0.13
	08/24/83	2430	8800	0.28	2590	8600	0.30
	08/25/83	2320	8800	0.26	2460	7800	0.32
	08/26/83	2360	5800	0.41	2420	5500	0.44
	08/28/83	1230	6800	0.18	1530	5800	0.26
	08/29/83	1260	7700	0.16	1130	7000	0.16
	08/30/83	1210	7100	0.17	943	7100	0.13
	08/31/83	2270	6800	0.33	2350	6100	0.39
	09/01/83	3180	6500	0.49	2440	7700	0.32
	09/02/83	2410	6100	0.40	1630	6100	0.27

line. Aniline will be dissociated or ionized at the pHs of the tests (pH 7 ± 1) and therefore may undergo ionic reactions with other polar cellular constituents. The proposed sorption isotherm Eqn. 70 probably does not apply to certain ionizable organics. Average values of sludge loadings and aqueous phase concentrations were used to project distribution coefficients using Eqn. 70. The results of this analysis are shown in Table 104.

TABLE 104. EVALUATION OF SORPTION EQUILIBRIUM EQUATION

Compound	Average loading (μg/g)	Average aqueous concentration (μg/L)	Observed K_{ba} (L/g)	Predicted K_{ba} (L/g)
Phenol	<70.2	<500	<0.14	<0.007
Aniline[a]	28.3	43	0.65	0.0018
Pentachlorophenol	1880	7280	0.26	29.29
Pentachlorophenate	1880	7280	0.26	1.71

[a] Analysis uses only data points for which C_a > detectable limit.

Pentachlorophenol sludge loading data for both the batch tests and the LAS study indicate that the aqueous phase concentration may be in the range where the sludge has achieved a saturation condition. A Langmuir isotherm constructed using data from both the batch and the continuous studies supports this observation. The Langmuir equation may be transformed to yield:

$$\frac{C_a}{C_s} = \frac{1}{ab} + \frac{1}{a} C_a \qquad \text{Eqn. 72}$$

A plot of the batch pentachlorophenol loading data and the average loading for each of the two continuous reactors is shown in Fig. 62. A similar analysis was conducted for batch data for 1,4-dichlorobenzene and for aniline data from the LAS systems. The results of these analyses are presented in Table 105. Each of these sets of data appear to fit the Langmuir model, as evidenced by the high correlation coefficients (r) obtained. However, only PCP data were available at concentrations both above and below a saturation value. Saturation concentrations in the aqueous phase related to the saturation loadings, a, in μg/g are 2 mg/L PCP, 21 g/L DCB, and 23 g/L for aniline. These somewhat tenuous values for aqueous phase saturation concentrations do not appear to be related to the K_{ow} of each compound.

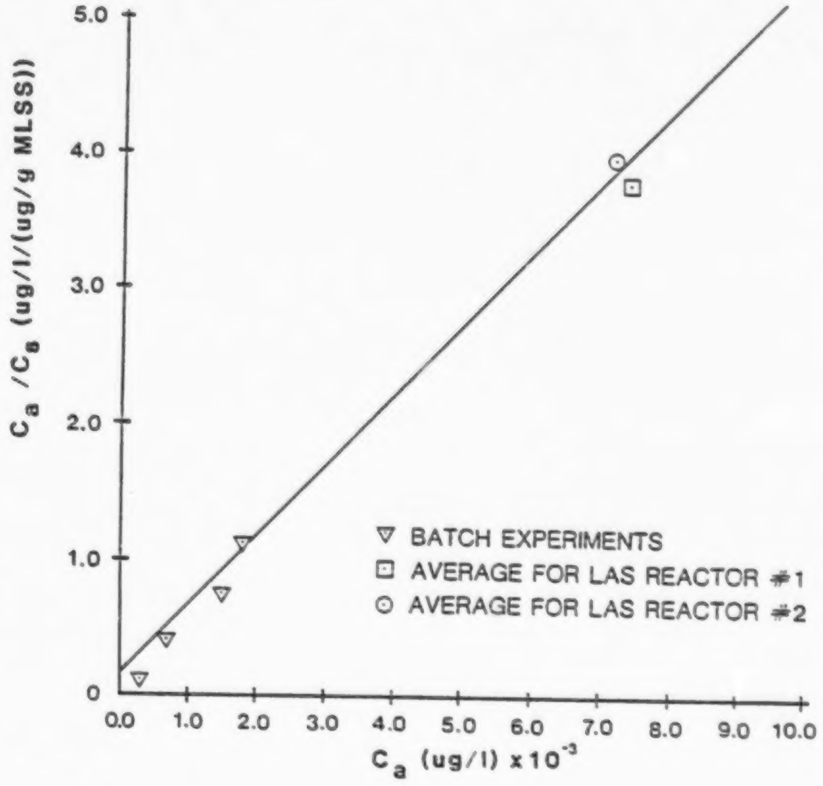

FIG. 62. LANGMUIR ISOTHERM PARAMETER ESTIMATION FOR PENTACHLOROPHENOL

TABLE 105. LANGMUIR ISOTHERM PARAMETERS

Compound	a[a]	b	Correlation coefficient for Eqn. 72
Pentachlorophenol	1908	8.52×10^{-3}	0.99
1,4-Dichlorobenzene	11560	8.48×10^{-5}	0.97
Aniline	42	5.73×10^{-2}	0.97

[a] Also equivalent to the maximum sludge loading or C_s in µg/g.

The distribution coefficients (K_{ba}) for low concentrations in the aqueous phase may be determined from the Langmuir equation as shown by Eqn. 69. The log (K_{ba}) obtained by this method was plotted versus log (K_{ow}) for PCP, DCB, and aniline. Data from other investigators and K_{ba} values calculated using Eqn. 71 were plotted on the same graph and are shown in Fig. 63.

Data reported by Pellazari are in the form of Freundlich K_f values. Average values of n for this data set were generally close to unity and may be neglected with the assumption that the log (Freundlich K) values on Fig. 63 may vary by ±50%.

Data on Fig. 63 indicate that predictive Eqn. 66 is in close agreement with the distribution coefficients for DCB, PCP, and phenol and with data reported by Matter-Muller.[6] This conclusion should be experimentally verified by performing sorption isotherm studies for compounds with a wide range of K_{ow} values. Aqueous phase concentrations should be varied over several orders of magnitude to verify the assumption that a linear relationship exists between K_{ow} and distribution coefficients at low concentrations and that a saturation effect does occur at relatively high aqueous phase concentrations.

Validation of Biological Oxidation Predictive Fate Methods

Mixed liquor samples from the LAS systems were analyzed by a static assay procedure at the University of Tennessee to determine mineralization rates. Procedures for conducting the mineralization assay are described in detail in Section 9. Initial concentrations used in the batch tests were 40, 130, and 100 mg/L for phenol, toluene, and aniline, respectively. Incubation times of 4, 24, and 8 hr were used for phenol, toluene, and aniline, respectively.

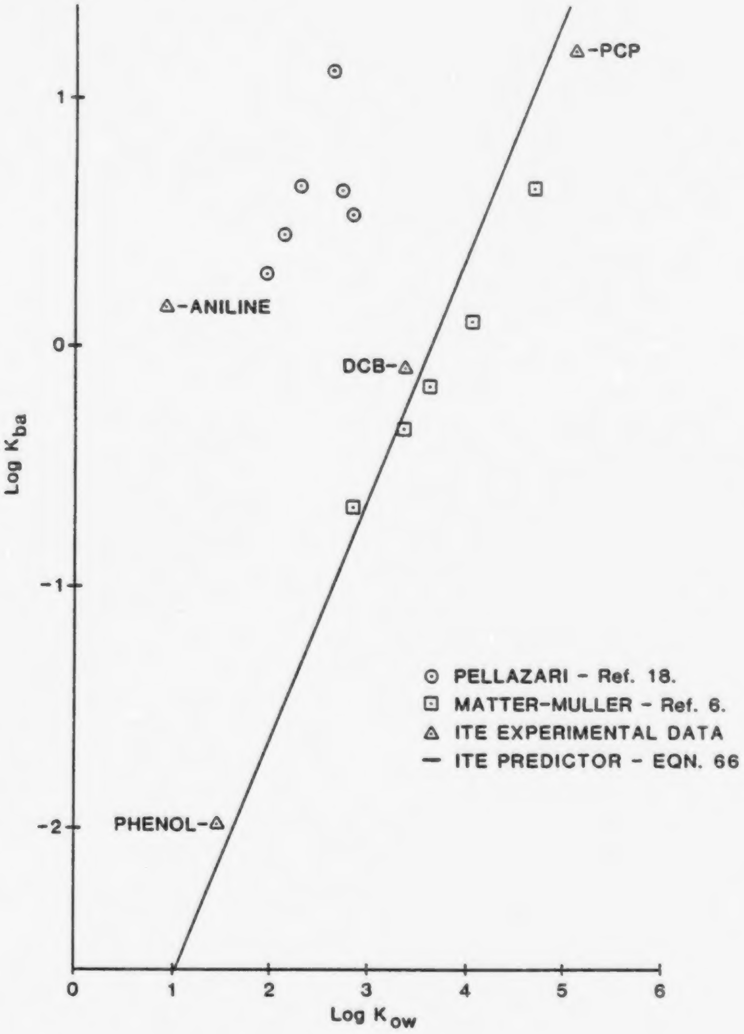

FIG. 63. LOG (K_{ba}) VERSUS LOG (K_{ow})

Mineralization rates were calculated by the following equation:

$$r^{bm} = \frac{f_m \cdot C_{ao}}{BHRT} \qquad \text{Eqn. 73}$$

where:

r^{bm} = biological mineralization rate (mg/L-hr of test compound),
f_m = fraction of feed ^{14}C converted to $^{14}CO_2$ in time t,
C_{ao} = concentration of test compound at start of incubation time, and
BHRT = incubation time (hr).

If mineralization is assumed to be a first order reaction, the mineralization rate constant may be calculated as follows:

$$K_1^{bm} = \frac{\ln \ (1/1-f_m)}{BHRT} \qquad \text{Eqn. 74}$$

Average mineralization rates and mineralization rate constants were also calculated for the LAS reactors using the material balance data presented in Tables 89 and 92. The mineralization rate in the continuous reactors was calculated as follows:

$$R_{BM} = \frac{f_m \cdot C_{ao}}{HRT} \qquad \text{Eqn. 75}$$

where:

f_m = fraction of feed ^{14}C converted to $^{14}CO_2$, and
HRT = hydraulic residence time (hr).

The first order mineralization rate constant for the complete mix continuous flow LAS reactors was calculated as follows:

$$K_1^{bm} = \frac{[1.0/(1.0-f_m)] -1}{HRT} \qquad \text{Eqn. 76}$$

The results of this analysis are presented in Table 106. Batch mineralization rates and rate constants are the averages for 5, 5, and 10 observations for each reactor for phenol, toluene, and aniline, respectively.

TABLE 106. COMPARISON OF MINERALIZATION RATES AND MINERALIZATION RATE CONSTANTS IN FILL-AND-DRAW AND CONTINUOUS FEED SYSTEMS

Compound	Reactor	MLSS (mg/L)	Fill-and-draw		Continuous (LAS)	
			Rate (mg/L•hr)	Rate constant K_1^{bm} (hr^{-1})	Rate (mg/L•hr)	Rate constant K_1^{bm} (hr^{-1})
Phenol	1	2220	0.75	1.95×10^{-2}	19.0	4.41×10^{-2}
	2	3650	0.40	1.02×10^{-2}	21.2	4.86×10^{-2}
Toluene	1	1280	0.63	5.15×10^{-3}	5.16	8.22×10^{-2}
	2	2370	0.55	4.46×10^{-3}	5.80	10.1×10^{-2}
Aniline	1	1690	4.10	4.97×10^{-2}	2.60	5.36×10^{-2}
	2	2550	3.50	4.10×10^{-2}	2.63	5.65×10^{-2}

Mineralization rates for phenol and toluene were found to be significantly lower in the batch reactors than in the continuous systems. The average concentration of the test compound in the feed varied from compound to compound, with average feed concentrations of 986, 195, and 109 mg/L for phenol, toluene, and aniline, respectively. The reactor aqueous concentrations were at the detection limit - a fraction of a mg/L. Even so, the mineralization rates are proportional to the feed concentrations instead of the reactor concentrations. Both phenol and aniline LAS systems were shown to have approximately 56% conversion of feed ^{14}C to $^{14}CO_2$; however, the mineralization rates vary approximately in proportion to the ratios of the feed concentrations of the respective test compounds. However, the mineralization rate constants are comparable on a quantitative basis and are relatively close for phenol and aniline for the continuous systems. The values of mineralization rate constants determined in the continuous systems would rank the three compounds in the order toluene>aniline>phenol in terms of the percent conversion of the parent compound to CO_2.

Mineralization rates obtained in the batch reactors were significantly lower for phenol and toluene than those obtained in the continuous systems while the mineralization rates for aniline were 57 and 33% higher in the batch tests than in the continuous tests for reactors 1 and 2, respectively. The depression of the mineralization rates observed in the batch reactors appears to contradict the conventional Monod model, in which biological oxidation rates increase with increasing concentration up to a maximum limiting rate. Phenol mineralization data presented in Section 9 indicated increasing rates of phenol mineralization at concentrations up to 120 mg/L.

Several factors may contribute to the differences in mineralization rates observed in the batch and continuous reactors. Initial concentrations of the test substrate were much higher in the batch tests than the concentrations in the mixed liquor in the continuous tests. If mineralization rates are truly first order, high mineralization rates would be expected at the concentrations employed in the batch reactors. If mineralization rates are zero order and independent of concentration, rates measured in the batch reactor would have been expected to more closely approximate those measured in the continuous systems. These observations lead to the conclusion that the initial phenol and toluene concentration used in the batch reactors may have been inhibitory to the biomass from the continuous reactors. Concentration differences in the batch and continuous systems may have also caused a shift in relative percentages of ^{14}C utilized in cellular growth and $^{14}CO_2$ production.

Rate studies on phenol disappearance yielded results similar to those observed for phenol mineralization. Batch tests conducted at initial phenol concentration of 30 mg/L yielded a phenol disappearance rate of 1.75 mg/L-hr over an 8-hr period while average phenol disappearance rates of approximately 36.5 mg/L-hr were observed in the two continuous reactors. However, kinetic disappearance rate constants, K_1^b, calculated for phenol range in the order of 0.1 hr^{-1} for the batch assay and 2100 hr^{-1} for the LAS system. Data from the phenol batch assay understated this disappearance rate constant by four orders of magnitude! The lower disappearance rate constants for the batch assay are corroborated in other experiments (see Fig. 44C).

The differences noted in the batch and continuous systems illustrate that batch assays must be conducted over a range of concentrations in order to provide predictive methods for mineralization rate kinetics for continuous systems. Also, experiments designed to determine the overall reaction order for the mineralization process should be incorporated into the assays. The major differences in disappearance rate constants suggest that batch assays may be inappropriate for determination of continuous disappearance kinetics. Further work is needed to clarify this area.

Data presented in Section 9 of this report indicated that the average ratio of organisms able to degrade the test compound to the total number of organisms for sludge samples from the LAS studies ranged from 6.7×10^{-6} to 1.6×10^{-3}. If the measured degradative organisms are assumed to be responsible for the conversion of the test compound at all steps along the metabolic pathway, these ratios would indicate extremely low growth yield coefficients for the degradative organisms. Alternatively, this data may be viewed as evidence for copious release of metabolites back into the mixed liquor and adjunct growth of organisms not possessing primary degradative capability for the initial substrate of interest.

Material balance calculations for the LAS study based upon the fate of feed ^{14}C appear to contradict the low growth yield coefficients which would be inferred from the measured ratio of degradative to total cells. Data to substantiate this conclusion are presented in Table 107. The percent conversion of feed ^{14}C to cell mass was calculated from the ratio of ^{14}C in waste solids plus ^{14}C in solids in the reactor at end of the run divided by the total feed ^{14}C. As shown in Table 107, the conversion of feed ^{14}C was very consistent for the three test compounds, with five of the six data points falling in the range of 26 to 29%. Measurements of MLSS samples

TABLE 107. CELL YIELDS FOR TEST COMPOUNDS BASED ON ^{14}C BALANCE

Compound	Reactor No.	Average ratio of test degraders to total cells	Percent conversion of test compound feed ^{14}C to cell mass ^{14}C	Test compound in feed (mg/day)	Calculated cell yield from conversion compound (mg/day)	Measured total cell yield (mg/day)
Phenol	1	2.7×10^{-3}	26.5	9,880	5,820	6,650
	2	2.8×10^{-3}	26.0	10,000	5,780	4,710
Toluene	1	1.4×10^{-3}	27.1	1,200	743	3,570
	2	7.8×10^{-6}	28.8	1,200	775	3,060
Aniline	1	1.2×10^{-4}	27.6	2,040	1,250	3,700
	2	9.1×10^{-5}	20.5	2,180	2,870	2,870

during the toluene and aniline studies indicated that the weight fraction of carbon in the MLSS was approximately 0.45. This value agrees well with values reported in the literature, which are normally around 0.5 g carbon per g of cell mass. If the assumption is made that the ratio of nonradiolabeled phenol carbon to cell mass is the same as the ratio of conversion of ^{14}C labeled phenol carbon to cell mass, the net growth of biomass on the test substrate can be predicted as follows:

$$DX = \frac{DF \cdot (PC/100)}{f_c} \qquad \text{Eqn. 77}$$

where:

DX = net growth of biomass (mg MLSS/day),
DF = conversion of test compound (mg/day),
PC = percent conversion of test compound carbon to cell carbon, and
f_c = weight fraction of carbon in MLSS.

The predicted net cell yields based on test compound conversion are shown in Table 107. Comparison of predicted net cell yields based on compound conversion and the measured cell yields indicates that a relatively high percentage of the cell mass is derived from the test substrate. The ratios of predicted cell yield to total measured cell yield is very close to the ratio of theoretical TOC in the feed test compound to the total feed TOC for aniline and toluene.

These results indicate that the population assay method that has been developed may measure the relative fraction of biological solids which are able to biotransform the test substrate. However, it appears that a much larger fraction of the biological population is able to grow on carbon derived from the test substrate after the substrate is broken down into soluble metabolites.

Problems and improvements in degrader population assay techniques are discussed in Section 9 of this report.

SECTION 11

DEVELOPMENT OF AN OVERALL MATHEMATICAL PREDICTIVE FATE METHOD

SUMMARY

An overall mathematical predictive fate method was developed in order to predict the equilibrium aqueous phase concentration in the secondary effluent from a complete-mix activated sludge system. The stripping PFM and the equilibrium sorption PFM for substrates at low concentrations are incorporated into traditional nonstructured biomass design models taken from environmental engineering.

Since no bio-oxidation PFM was determined in this study, consideration of the biomass as a single, non-conservative reactant was the only approach that could be justified. Development of multiple component biomass models or structured-biomass models must be left for future projects. Here, substrate bio-oxidation disappearance rates must be determined from existing experimental data corrected for stripping and sorption. However, the authors believe that further work on the bio-oxidation PFM may result in a predictive capability for the specific degrader population and a two- (or more) component bio-oxidation model may be successfully incorporated. Further work resolving the sorptive (desorptive) linkages between the two populations as well as incorporation of the sorption saturation effect will be needed to support a more detailed model.

Budget limitations late in the project precluded the testing at the LAS scale of a compound suspected of having significant stripping, sorption, and bio-oxidation mechanisms. LAS studies with 1,4-dichlorobenzene were initially planned, but later abandoned in order to more fully resolve some questions in the bio-oxidation PFM development. Data from this run could have been used to test the overall PFM discussed in this section. As it is, equations presented in this section must be considered only as a proposed method and entirely unvalidated at present.

Further work on the overall PFM should include testing with a substrate incorporating tendencies towards all three transport/conversion mechanisms, as well as continued refinement as more work is done in the sorption and bio-oxidation PFMs. Ultimately, this model and future modifications can be adapted for use in plug-flow plants and conversion to a dynamic simulation would allow eventual treatment of the transient effects experienced in full-scale activated sludge treatment systems.

Other specific recommendations follow:

- Develop methodology of predicting bio-oxidation disappearance rates from simple tests or compound physical chemical properties.
- Continue work on the effects of alternative substrates on the bio-oxidation rate of a given compound.
- Investigate the saturation effect observed for sorption of organic compounds to biological solids. Investigate approaches for predicting saturation loadings from physical properties.
- Implement additional work on competitive sorption mechanisms and the effect on the equilibrium loading of a given compound.
- Verify the stripping predictive fate method for other geometric configurations, hydraulic regimes, and diffusion system types.

OVERVIEW

Two approaches were considered for the development of a mathematical model of the fate of a specific compound in the activated sludge process. The two conceptual models which were considered differed in the method in which the biological population was determined.

The proposed basis for the first conceptual model was to identify and quantify the fraction of the biological population that was capable of degrading a given compound and to develop kinetic expressions based upon the measured population of specific compound degraders. The assay method which was developed for quantifying specific compound degraders indicated that very small fractions of the total biomass (6.7×10^{-6} to 1.6×10^{-3}) were actively involved in the initial conversion of the parent compound. If sorption is assumed to be the first step in the bio-oxidation process, further development of this type of model will require development of techniques for determining if sorption occurs uniformly for the entire biological population or if there is a difference in the sorption mechanism between specific compound degraders and the remainder of the biological population. Interchange of the specific compound between the two biomass populations (degraders and nondegraders) also must be elucidated.

An alternative conceptual model may be developed by expressing the rate equations in terms of the entire biological population expressed as total organisms per mL, MLSS, MLVSS, or other suitable measures of biological population density. Development of this type of model for the fate of a specific compound must be coupled with a model describing microbial growth kinetics in terms of a non-specific parameter (BOD, COD, or TOC), since only a fraction of the total biological growth may be attributed to the biological conversion of the specific compound.

MATHEMATICAL MODELS IN TERMS OF NON-SPECIFIC PARAMETERS (BOD, TOC, and COD)

The development of a model using the second approach may utilize the conventional design equations developed by Lawrence and McCarty for describing biological growth in terms of a non-specific substrate parameter (BOD, COD, or TOC).[102] These equations utilize an empirically developed relationship between growth and a non-specific substrate utilization as follows:

$$\frac{dX}{dt} = Y\frac{dF}{dt} - k_d X \qquad \text{Eqn. 78}$$

where:

$\frac{dX}{dt}$ = net growth rate of microorganisms, wt/volume-time,

Y = growth-yield coefficient, wt of microorganisms/wt of substrate utilized,

$\frac{dF}{dt}$ = rate of substrate utilization by microorganisms, wt/volume-time,

k_d = microorganisms-decay coefficient, time^{-1}, and

X = concentration of microorganisms, wt/volume.

The rate of substrate utilization has been described by an equation similar to the Monod equation as follows:

$$\frac{dF}{dt} = \frac{k_b^{max} \cdot X \cdot C_{ae}}{K_s + C_{ae}} \qquad \text{Eqn. 79}$$

where:

k_b^{max} = maximum rate of waste utilization per unit weight of microorganisms, time^{-1},

K_s = waste concentration at which rate of waste utilization per unit weight of microorganisms is one-half the maximum rate, wt/volume, and

C_{ae} = concentration of waste surrounding the microorganisms, wt/volume.

In the limiting case when C_{ae} is much greater than K_s, K_s can be neglected in the denominator of Eqn. 79, yielding an expression that is zero order with respect to substrate concentration. In the second limiting case, when K_s is much greater than C_{ae}, Eqn. 79 reduces to an expression that is first order with respect to substrate concentration:

$$\frac{dF}{dt} = K^1 \cdot X \cdot C_{ae} \qquad \text{Eqn. 80}$$

where:

$K^1 = \frac{k_b^{max}}{K_s}$ = substrate utilization rate constant, volume wt^{-1} $time^{-1}$.

Equations 78, 79, and 80 have been applied to biological wastewater treatment systems with the assumption that all substrate removal is attributed to biological action. However, for a waste stream containing significant amounts of substrate which may be stripped or sorbed, these equations must be modified to account for substrate lost through these mechanisms. Stover has proposed that biological kinetic coefficients should be developed after subtracting the equivalent amount of BOD, TOC, or COD lost through other mechanisms from the influent concentration.[20] If this approach is carried a step further, the quantity of substrate lost via the stripping and sorption mechanism must also be related to the system parameters affecting these mechanisms (air flow rate, tank geometry, etc.).

Equation 79 is normally preferred over Eqn. 80 since it describes the change in the order of the bio-oxidation rate from a first order rate to a zero order rate as a function of the effluent substrate concentration while Eqn. 80 describes only a first order rate process. However, derivation of reliable estimates of the parameters k_b^{max} and K_s normally requires operation of 4-6 reactors at a wide range of θ_c values. Due to budgetary and time constraints, reactors were operated at two values of θ_c in this study. Therefore, due to the limited data available, Eqn. 80 was chosen to model the bio-oxidation process rather than Eqn. 79.

The biological kinetic coefficients Y, k_d, and K^1 in terms of TOC were developed for the four LAS studies. The TOC in the influent was adjusted by subtreating the theoretical TOC which was lost through the stripping and sorption mechanisms. Y and k_d were derived by dividing both sides of Eqn. 78 by X and plotting (dX/dt)/X versus (dF/dt/X) for the experimental data. Y is the slope of the resulting plot and k_d is the y-axis intercept.

By writing Eqn. 80 on a finite mass and time basis, the following expression can be developed for K^1:

$$K^1 C_{ae} = \frac{Q_a (C_{ao} - C_{ae})}{X V} \qquad \text{Eqn. 81}$$

where:

Q_a = influent flow (L/day),

C_{ao} = influent TOC concentration adjusted for stripping and sorption losses (mg/L), and

V = reactor volume (L).

K^1 was derived for each of the studies by plotting the left side of Eqn. 76 versus C_{ae}, with K^1 being the slope of the resulting line. The results of the analysis of the biokinetic coefficients Y, k_d, and K^1 in terms of TOC are presented in Table 108.

TABLE 108. BIOKINETIC CONSTANTS IN TERMS OF TOC

Compound	Growth yield coefficient, Y (mg MLSS/mg TOC)	Microorganism decay constant, k_d (hr^{-1})	Substrate utilization rate constant, K^1 (L/mg-hr)
Phenol	0.68	3.33×10^{-3}	6.99×10^{-5}
Toluene	0.84	1.25×10^{-3}	6.67×10^{-5}
Aniline	1.51	4.17×10^{-3}	5.24×10^{-5}
Pentachlorophenol	1.33	4.58×10^{-3}	4.56×10^{-5}

Design equations for a complete mix activated sludge system with cellular recycle have been derived in several references.[103,104] Those equations are general and may be used with data either corrected or uncorrected for abiotic mechanisms. It can be shown that C_{ae} and X can be calculated for this system as follows:

$$C_{ae} = \frac{1/\theta_c + k_d}{YK^1} \qquad \text{Eqn. 82}$$

$$X = \frac{Y\,Q_a(C_{ao} - C_{ae})}{V\,(1/\theta_c + k_d)} \qquad \text{Eqn. 83}$$

where:

X = concentration of biological solids in the reactor (mg/L of MLSS),

Q_a = influent flow (L/day),

C_{ao} = influent TOC (mg/L),

C_{ae} = effluent TOC (mg/L),

V = reactor volume (L),

k_d = microorganism decay coefficient (day^{-1}),

θ_c = mean cell residence time (days),

Y = growth yield coefficient (mg MLSS/mg TOC,, and

K^1 = substrate utilization rate constant (L/mg-hr).

Mathematical Model in Terms of a Specific Compound --

The kinetic model which is proposed to describe the fate of specific compounds in the activated sludge process was developed on the basis of steady-state conditions within the treatment system. Assumptions required in the development of the model are stated in order to understand the limitations of the proposed model. A diagram of a typical complete-mix activated sludge process for which the model will be developed is shown on Fig. 64.

Most design kinetic models in terms of non-specific parameters are based upon a material balance for a soluble substrate in the aqueous phase. A material balance around the system for a specific compound in the aqueous phase may be written as follows:

$$-V\left(\frac{dC_{ae}^{tot}}{dt}\right) = Q_a\,C_{ao} - (Q_a - Q_w)\,C_{ae} - Q_w\,C_{ae} - V\left(\frac{dC_{ae}^{sp}}{dt}\right) - V\left(\frac{dC_{ae}^{b}}{dt}\right) - V\left(\frac{dC_{ae}^{st}}{dt}\right) \qquad \text{Eqn. 84}$$

where:

V = aeration basin volume (L),

$\frac{dC_{ae}^{tot}}{dt}$ = total net rate of change of soluble concentration of substrate in aeration basin (mg/L-day),

Q_a = influent flow (L/day),

Q_w = waste sludge flow (L/day),

C_{ao} = soluble concentration of substrate in influent (mg/L),

C_{ae} = soluble concentration of substrate in effluent (mg/L),

$\frac{dC_{ae}^{sp}}{dt}$ = net rate of change of soluble concentration of substrate in aeration basin due to sorption to biological solids (mg/L-day),

$\frac{dC_{ae}^{b}}{dt}$ = net rate of change of soluble concentration of substrate in aeration basin due to bio-oxidation (mg/L-day), and

$\frac{dC_{ae}^{st}}{dt}$ = net rate of change of soluble concentration of substrate in aeration basin due to stripping (mg/L-day).

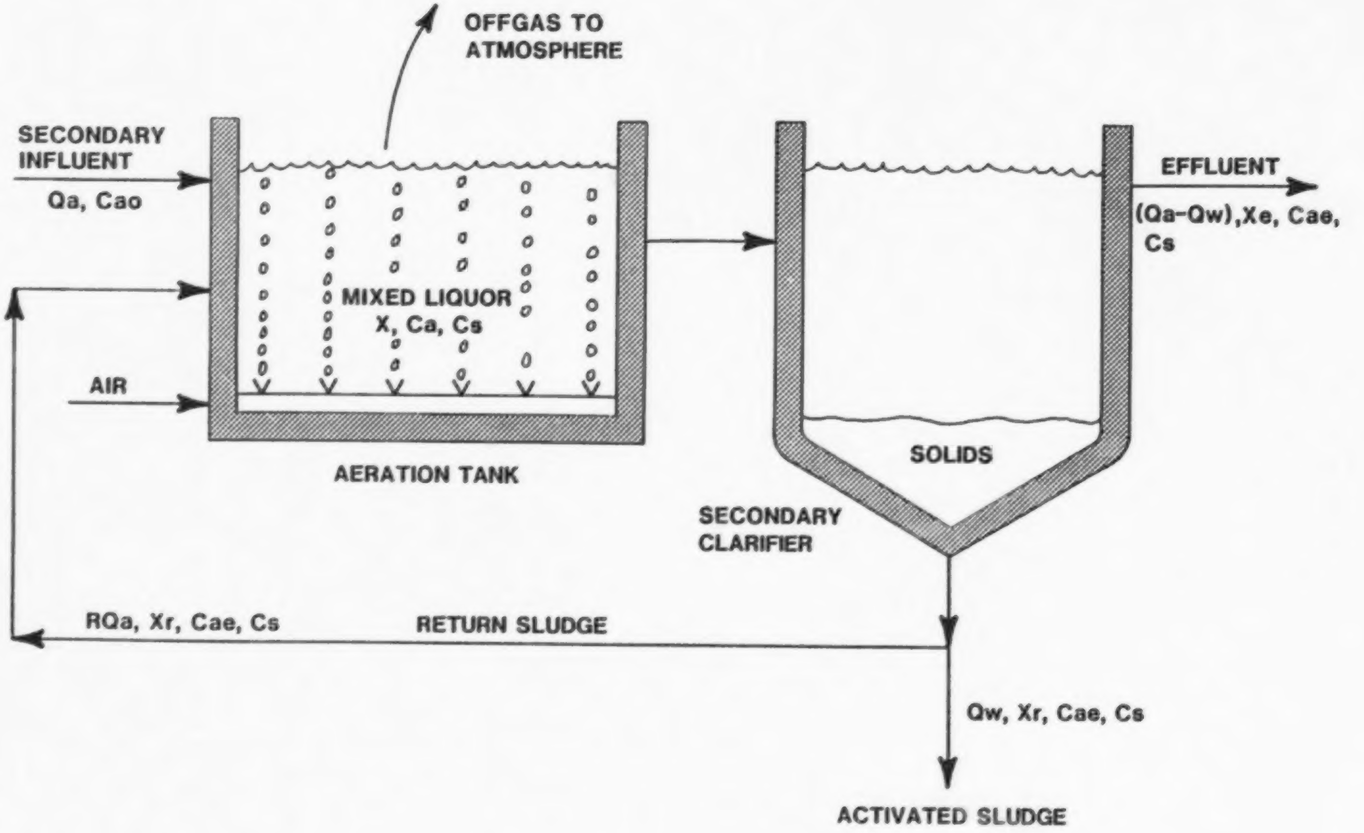
OFFGAS TO
ATMOSPHERE
SECONDARY
INFLUENT
Qa, Cao
AIR
MIXED LIQUOR
X, Ca, Cs
AERATION TANK
EFFLUENT
(Qa–Qw),Xe, Cae,
Cs
SOLIDS
SECONDARY
CLARIFIER
RQa, Xr, Cae, Cs
RETURN SLUDGE
Qw, Xr, Cae, Cs
ACTIVATED SLUDGE

FIG. 64. MODEL ACTIVATED SLUDGE PROCESS

Development of Eqn. 84 is based on the following assumptions:[102]

1. Complete mixing is achieved in the aeration tank.
2. Soluble influent substrate concentration remains constant.
3. All substrate in the influent is in the soluble form.
4. No microbial solids are contained in the raw wastewater to the aeration tank.
5. No bio-oxidation, stripping or sorption occurs in the secondary clarifier.
6. No sludge accumulates in the secondary clarifier.
7. Steady-state conditions prevail throughout the system.
8. No saturation effect is present regarding biomass sorption.

Since the rate of change of the soluble substrate concentration due to stripping is equal to the air flow rate times the concentration of the substrate in the air and the net rate of change of soluble substrate due to sorption is equivalent to the rate at which biological solids are removed from the system times the substrate loading on the solids, a material balance for the substrate in all phases can be written for the system as:

$$-V\left(\frac{dC_{ae}^{tot}}{dt}\right) = Q_a\,C_{ao} - \left[Q_a - Q_w\right] C_{ae} - Q_w\,C_{ae} - \left[Q_a - Q_w\right] X_e\,C_s - Q_w X_r C_s - Q_{air} C_{air} - V\left(\frac{dC_{ae}^{b}}{dt}\right) \qquad \text{Eqn. 85}$$

where:

C_s = substrate loading on biological solids (g substrate/g solids),

X = concentration of biological solids in aeration basin (mg/L),

X_e = concentration of biological solids in effluent (mg/L),

X_r = concentration of biological solids in recycle sludge stream (mg/L),

Q_{air} = air flow rate (L/day), and

C_{air} = substrate concentration in exhaust air (mg/L).

At steady state, the term dC_{ae}^{tot}/dt in Eqn. 85 becomes zero. The solids capture efficiency approaches 100% for a properly operating secondary clarifier, the term $(Q_a - Q_w)\,X_e\,C_s$ becomes negligible and drops out of Eqn. 84 and the following expression may be derived for Q_w:

$$Q_w = \frac{XV}{\theta_c X_r} \qquad \text{Eqn. 86}$$

X_r may be defined in terms of the sludge volume index (SVI) as:

$$X_r = \frac{10^6}{SVI} \qquad \text{Eqn. 87}$$

The rate of mass removal by stripping, $V\,(dC_{ae}^{st}/dt)$ in Eqn. 84 is equivalent to the term $Q_{air}\,C_{air}$ in Eqn. 85. A predictive fate method for estimating the rate of stripping has been presented and discussed in Sections 5 and 9. This equation assumes first order and is of the form:

$$V\left(\frac{dC_{ae}^{st}}{dt}\right) = V\,K_a^{st}a\,C_{ae} \qquad \text{Eqn. 88}$$

where:

$K_a^{st}a$ = stripping rate constant (day^{-1}),

the stripping rate constant was shown to be related to the Henry's law constant, the air flow rate and reactor volume were as follows:

$$K_a^{st}a = \frac{Q_{air}}{V} \cdot 6.18 \times 10^{-5}\,H_c^{1.045} \qquad \text{Eqn. 89}$$

where:

Q_{air} = air flow (L/day),

H_c = Henry's law constant (torr • L/g-mole), and

V = reactor volume (L).

Equation 89 differs from the equation previously presented, Eqn. 62, in that the constant term has been adjusted to reflect the change in units in Q_{air} and $K_a^{st}a$.

The rate of mass removal by sorption, $V\,(dC_{ae}^{sp}/dt)$ in Eqn. 84 is equivalent to the sum of the terms $[(Q_a-Q_w)X_eC_s]$ and $[Q_wX_rC_s]$ in Eqn. 85. If X_e is assumed to be negligible, $V\,(dC_{ae}^{sp}/dt) = [Q_wX_rC_s]$.

As shown in Section 8, sorption appears to reach an equilibrium condition very quickly (at least for lower octanol-water partition coefficient compounds) and the sorption rate constants which were determined could be considered as only a lower bound on these values. For a complete-mix activated sludge process with a hydraulic retention time of several hours, sorption of specific substrates to biological solids may be modeled as an equilibrium process. However, this assumption may not be valid for other hydraulic regimes (plug flow) or higher octanol-water partition coefficient compounds that might require a longer time to achieve equilibrium.

Assuming equilibrium between the substrate in the aqueous and solid phases, C_s may be related to C_{ae} by an sorption isotherm. It was shown in Sections 8 and 10 that a Langmuir isotherm equation appears to be satisfactory for describing the sorption equilibrium between aqueous and solid phases over a broad aqueous phase concentration range; however, no predictive method currently exists for estimating the parameters of the Langmuir isotherm equation. An isotherm equation was also proposed which could be derived in terms of the octanol-water partition coefficient (K_{ow}) for compounds with moderate to high K_{ow} values and for the range of aqueous phase concentrations where a linear relationship exists with loading on biological solids. This equation is of the form:

$$C_s = \frac{K_{ow} \cdot f_L \cdot C_{ae}}{\rho_L} \qquad \text{Eqn. 90}$$

where:

K_{ow} = octanol-water partition coefficient (concentration ratio),

f_L = lipid weight fraction of biological solids (μg lipid/μg dry solids),

ρ_L = density of lipids (g/L),

C_s = equilibrium substrate loading on solids (μg/g), and

C_{ae} = equilibrium substrate concentration in aqueous phase (μg/L).

Converting units of the parameters in Eqn. 90 to the units required in Eqn. 85, Eqn. 90 becomes:

$$C_s = \frac{K_{ow}\ f_L\ C_{ae}}{1000\rho_L} \qquad \text{Eqn. 91}$$

where:

C_s = equilibrium substrate loading on solids (g/g) and

C_{ae} = equilibrium substrate concentration in aqueous phase (mg/L).

The term $dC_{ae}{}^{b}/dt$ in Eqn. 84 is equivalent to the term dF/dt in Eqn. 80 where K^1 is the substrate utilization rate constant for the specific compound. K^1 values for the specific compounds used in the LAS may be derived from plotting the experimental data in the form specified by Eqn. 81 after adjusting C_{ao} for stripping and sorption losses. K^1 rates determined for the LAS studies must be considered to be minimum values, since concentrations in the effluents were below detectable limits. K^1 values (L/mg-day) determined for the LAS studies are as follows:

Phenol	$>7.61 \times 10^{-1}$
Toluene	>2.54
Aniline	$>6.70 \times 10^{-1}$
Pentachlorophenol	0.00

THE OVERALL MATHEMATICAL PREDICTIVE FATE METHOD

For a given set of design conditions and with known values of Y, K^1, and k_d for a non-specific parameter, the fate of a specific compound in an activated sludge process may be assessed by making appropriate substitutions from Eqns. 80, 86, 87, 88, 89, and 91 into Eqn. 85. Equation 85 may then be solved for C_{ae} as follows:

$$C_{ae} = \frac{Q_a C_{ao}}{Q_a + \dfrac{XV\,K_{ow} f_L}{1000 \rho_L \theta_c} + VK_a{}^{st}a + VK^1X} \qquad \text{Eqn. 92}$$

A set of example calculations is presented for assessing the fate of phenol for the following conditions:

$V = 11$ L
$Q_a = 11$ L/day
$Q_{air} = 2880$ L/day
$C_{ao} = 1334$ mg/L TOC
$C_{ab} = 985$ mg/L phenol
$Y = 0.68$ mg MLSS/mg TOC
$K_d = 0.08$ day^{-1}
$K^1 = 1.67 \times 10^{-3}$ L/mg-day (TOC)
$K^1 = 7.61 \times 10^{-1}$ L/mg-day (phenol)
$\theta_c = 5$ days
$f_L = 0.20$
$\rho_L = 900$ g/L
$K_{ow} = 30.9$
$H_c = 0.25$

Calculate effluent TOC using Eqn. 82:

$$C_{ae} = \frac{(1/5 + 0.08)}{(0.68)(1.67 \times 10^{-3})} = 247 \text{ mg/L}$$

Calculate MLSS using Eqn. 83:

$$X = \frac{(0.68)(11)(1334 - 247)}{(11)(1/5 + 0.08)} = 2640 \text{ mg/L}$$

Calculate stripping rate constant using Eqn. 89:

$$K_a{}^{st}a = \frac{2880}{11} \cdot 6.18 \times 10^{-5} \cdot (0.25)^{1.045} = 0.0038 \text{ day}^{-1}$$

Calculate effluent phenol concentration using Eqn. 92:

$$C_{ae} = \frac{(11)(985)}{11 + \frac{(2640)(11)(30.9)(0.2)}{(1000)(900)(5)} \quad (11)(0.0038) + (11)(2640)(0.761)}$$

$$C_{ae} = 0.49 \text{ mg/L}$$

The calculated value of C_{ae} may then be substituted into Eqn. 88 to determine the mass stripping rate as follows:

$$V \cdot K_a{}^{st}aC_{ae} = (11)(0.0038)(0.49) = 0.021 \text{ mg/day}$$

The compound loading on biological solids may be calculated from Eqn. 91.

$$C_s = \frac{(30.9)(0.20)(0.49)}{(1000)(900)} = 3.36 \times 10^{-6} \text{ g phenol/g solids}$$

SPECIFIC COMPOUND PREDICTIVE FATE EQUATIONS

By dividing the numerator and denominator of the right side of Eqn. 92 by Q_a and rearranging, equations may be developed to estimate the percent of substrate fed to the system (1) remaining in the effluent, (2) sorbed to biological solids, (3) stripped from the system, and (4) biologically degraded as functions of important design variables for a given plant.

If:

$$A = \frac{HRT \times K_{ow}\ f_L}{1000\ \rho_L \theta_c} \qquad \text{Eqn. 93}$$

$$S = HRT\ \frac{Q_{air}}{V}\ 6.18 \times 10^{-5}\ H_c{}^{1.045} \qquad \text{Eqn. 94}$$

$$B = HRT\ K^1X \qquad \text{Eqn. 95}$$

Then:

$$REM_{sp} = \frac{A}{1 + A + S + B} \cdot 100 \qquad \text{Eqn. 96}$$

$$REM_{st} = \frac{S}{1 + A + S + B} \cdot 100 \qquad \text{Eqn. 97}$$

$$REM_{b} = \frac{B}{1 + A + S + B} \cdot 100 \qquad \text{Eqn. 98}$$

$$REM_{e} = \frac{1}{1 + A + S + B} \cdot 100 \qquad \text{Eqn. 99}$$

where:

HRT = hydraulic residence time (days),

X = biomass concentration (mg/L),

K_{ow} = octanol-water partition coefficient (unitless concentration ratio, wt/volume),

f_L = lipid wt faction in the biomass,

ρ_L = lipid density (g/L),

θ_c = mean cell residence time in the system (days),

Q_{air} = air flow in the system (L/day),

V = system hydraulic volume (L),

H_c = Henry's law constant (Torr • L/g-mole),

K^1 = substrate utilization rate constant (L mg^{-1} day^{-1}), and

REM_{sp}, REM_{st}, REM_b, REM_e, = percent removals of fed substrate by sorption, stripping, biotransformation, and effluent mechanisms, respectively.

Note that all assumptions earlier stated to be applicable to the derivation of Eqn. 92 are also applicable to Eqns. 93 to 99.

Since no satisfactory method is available to predict K^1, one option is to apply Eqn. 92 to a given set of experimental data from a diffused air complete-mix actual sludge system to solve for K^1 or the product K^1X. One source of such data is, again, the Kincannon and Stover work.[4,19,20]

The use of these predictive equations may be demonstrated by assigning most probable values to the variables presented and also establishing reasonable ranges for the system-related variables. Assumed values are presented in Tables 109 and 110. A range for the variables K^1X is given that assumes fluctuations in the disappearance rate constant may be over three orders of magnitude. This is consistent with observations on toluene in our LAS work.

TABLE 109. ASSUMED VARIABLE VALUES AND RANGES FOR THE PREDICTIVE FATE EQUATIONS

Variables	Units	Most probable	Minimum	Maximum
HRT	Days	1	0.5	3
X	mg/L MLSS	2000	500	4000
f_L	Unitless wt fraction	0.2		
ρ_L	g/L	900		
θ_c	Days	2.5	0.5	15
Q_{air}/V	Day^{-1}	144	72	1440

TABLE 110. VALUES FOR K_{ow}, H_c, AND K^1X

Compound	K_{ow}	H_c	Most probable K^1X^a	Minimum K^1X^b
Phenol	31	0.25	50,000	50
Toluene	537	2769	13,200	13.2
Pentachlorophenol	7700	1.6	120	0.12

[a] Values derived from references 4, 19, 20.

[b] Minimum K^1X = 0.001 (most probable K^1X).

Using Eqns. 93 to 99 and selecting the worst and best cases of the ranged variables will allow the calculation of both a most probable value for such removal mechanisms and an evaluation of how removals may range in various real-world processes. Table 111 presents calculations assuming that biotransformation proceeds between the rate constant values shown in Table 110.

Sometimes upset conditions or the presence of toxic or inhibitory compounds disrupts the biotransformation process completely. These cases, too, may be quantified assuming that the biological disappearance rates K^1X equal zero. The fate mechanisms when no biotransformation for the compound of interest is underway are presented in Table 112.

These predictions reveal some interesting results. First, when biological disappearance rates approximate those found in Kincannon and Stover's work[4,19,20] (these could be considered optimal cases because of the steady-state nature of the feed concentrations and flow rates and the fact that they are representative of lab-scale treatability studies), all three compounds would exceed 98% biodegradation (most probable cases from Table 111). However, as reduced biological disappearance rates and the effects of plant designs are considered, wide variability in the fates can be expected.

TABLE 111. PREDICTED PERCENT REMOVALS OF PHENOL, TOLUENE, AND PENTACHLOROPHENOL ASSUMING ACTIVE BIOTRANSFORMATION[a]

Compound	Removal mechanism	Mechanism removal (%)	
		Most probable	Range
Phenol	Sorption	<1	<1 - 14
	Stripping	<1	<1 - 6
	Biotransformation	99.2	4.7 - 99.7
	Effluent	<1	<1 - 94.3
Toluene	Sorption	<1	<1 - 15
	Stripping	<1	<1 - 99.3
	Biotransformation	99.7	<1 - 99.9
	Effluent	<1	<1 - 10
Pentachlorophenol	Sorption	1.1	<1 - 97.5
	Stripping	<1	<1 - 28.6
	Biotransformation	98.1	<1 - 99.7
	Effluent	<1	<1 - 91.5

[a] Subject to the assumptions and derivations in this section.

TABLE 112. PREDICTED PERCENT REMOVALS OF PHENOL, TOLUENE, AND PENTACHLOROPHENOL ASSUMING NO BIOTRANSFORMATION[a]

Compound	Removal mechanism	Mechanism removal(%)	
		Most probable	Range
Phenol	Sorption	<1	<1 - 14
	Stripping	<1	<1 - 6
	Effluent	99.7	81 - 94.3
Toluene	Sorption	<1	<1 - 23
	Stripping	97.0	70 - 99.9
	Effluent	2.8	<1 - 10
Pentachlorophenol	Sorption	57	2 - 97.6
	Stripping	<1	<1 - 30
	Effluent	43	2 - 96.8

[a] Subject to the assumption and derivations in this section.

Fate of phenol can vary from essentially all biotransformed to all wasted in the effluent. Fate of toluene fluctuates between biotransformation and stripping and fate of pentachlorophenol can be split between sorption, biotransformation, and being wasted in the effluent with stripping being significant in some cases. When the biotransformation mechanism is inactive, as in the cases of plant upsets, transient conditions, or under toxic or inhibitory stress, phenol will remain in the effluent, toluene will strip, and pentachlorophenol will be split between sorption and the effluent. However, system design variables can still exert significant influence, particularly in the case of pentachlorophenol (Table 112).

Results from our laboratory-scale activated sludge study generally agree with the most probable values from Table 111 for phenol and for toluene under optimal operation. Toluene average values were approximately 82% biotransformed and 12% stripped. These values reflect the behavior of the system during optimal and upset periods, and the average values are consistent with the ranges given in Table 111. LAS data for pentachlorophenol indicate 6 to 8% PCP sorption and the remainder in the effluent. This is much lower than the most probable value in Table 112 and is felt to be the result of a saturation sorption process. The predictive equations presently only apply to concentrations below any saturation values but may be modified to include saturation sorption if concentrations exceed the minimum saturation value. Since little conclusive information on the saturation concentrations is available, only the non-saturated sorption Eqn. 93 is proposed here.

Strier[105] has proposed fate predictive equations for activated sludge treatment systems based on structure-activity relationships. These predictions for phenol, toluene, and pentachlorophenol are given in Table 113. If one compares the results from the fate equations in this section with those proposed by Strier, an interesting finding emerges. First, predictions on Table 113 for compound removed in the effluent generally agree with both the ranges and most probable values from Table 111, the results from Eqns. 93 to 99. However, the prediction of the specific fates in Table 113 (sorption, stripping, and biotransformation) vary substantially from the most probable values in Table 111. The ranges in Table 111 do generally encompass the ranges shown in Table 113. It is encouraging that results from two substantially different approaches to fate prediction (semi-deterministic -- this work, and stochastic -- Strier's[105]) do show some commonality.

One of the problem areas in biological waste treatment has been the apparent variability of wastes and their treatment efficiencies. After further verification, Eqns. 93 to 99 may offer some hope that much of the variability may be manageable and the magnitude of variation due to biological kinetic effects may be quantified.

TABLE 113. PREDICTIONS ON COMPOUND FATES USING UNVERIFIED STRUCTURE - ACTIVITY RELATIONSHIPS PROPOSED IN OTHER WORK[a]

Compound	Removal mechanisms	Mechanism removal (%)
Phenol	Sorption	5 - 10
	Stripping	5 - 10
	Biotransformation	80
	Effluent	0 - 10
Toluene	Sorption	10 - 20
	Stripping	20 - 40
	Biotransformation	50 - 55
	Effluent	0 - 20
Pentachlorophenol	Sorption	50 - 60
	Stripping	5 - 10
	Biotransformation	30 - 35
	Effluent	0 - 10

[a] From Ref. 105.

These results also agree with general findings collected in full-scale treatment plant surveys such as the "EPA-40 Plant Study."[100] It is now not difficult to accept that treatability for specific trace compounds is strongly influenced by changes in the instantaneous biological disappearance kinetics within a given process and by system design variables from plant to plant. This is particularly true for compounds with two strong fate mechanism tendencies like biotransformation and stripping. Wide variations between lab and pilot treatability studies and full-scale operation experienced by many practicing environmental engineers might also be understood in the context of the predictive equations presented.

Finally, the true variation in biological kinetics may be addressed and the more basic causes of the biological disappearance rate variations may be more closely determined after corrections for these other variations are made.

CONCLUSIONS

The mathematical model which has been developed provides a framework for the assessment of the fate of specific organic compounds in biological treatment processes. The proposed predictive fate methods allow the estimation of sorption and stripping losses from physical properties of the compound and measurable system parameters. However, additional research must be conducted in the following areas to complete the development of an overall predictive fate method:

- Develop methodology of predicting bio-oxidation disappearance rates from simple tests or compound physical chemical properties. Resolve the major variations seen in disappearance rate assays.

- Continue work on the effects of alternative substrates on the bio-oxidation rate of a given compound.

- Investigate the saturation effect observed for sorption of organic compounds to biological solids. Develop methodology for predicting saturation loadings from physical properties.

- Implement additional work on competitive sorption mechanisms and the effect on the equilibrium loading of a given compound.

- Verify the stripping predictive fate method for other design configurations, hydraulic regimes, and diffusion system types.

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APPENDIX

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